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* 10,332 volunteer abstracts, 17 symposia abstracts, 17 history of neuroscience abstracts, and 39 teaching of neuroscience abstracts.
1994 Program Committee

Irwin B. Levitan, Ph.D.
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Brandeis University

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SUNY, Stony Brook

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University of California, Berkeley

Lynn T. Landmesser, Ph.D., ex officio
Case Western Reserve University
School of Medicine
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92. Neuroscience Implications of Inborn Errors of Metabolism  
*Chaired by: A. DIAMOND* | 210 |
93. Molecular Mechanisms Controlling K+ Channel Diversity and Distribution in the Nervous System  
*Chaired by: J.M. NERBONNE* | 210 |

**History of Neuroscience Lecture—1:00 p.m.**

94. Evolving Concepts of Function of the Neocortex  
V.B. MOUNTCASTLE | No Abstract |

**Special Lecture—4:15 p.m.**

95. The Molecular Biology and Biophysics of Prions Causing CNS Degeneration  
S. PRUSINER | No Abstract |

**Slide Sessions—1:00 p.m.**

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Animals in Research Panel—5:30 p.m.

Presidential Symposium—8:00 p.m.
182. The Biology of Memory: From Synapses to Systems
Genes, Synapses, and the Molecular Switch for Long-term Memory
E.R. KANDEL | No Abstract
Brain Substrates of Basic Associative Learning
R.F. THOMPSON | No Abstract
Traces of Recognition
M. MISHKIN | No Abstract
Neuromodulatory Systems and Regulation of Memory Storage
J.L. MCGAUGH | No Abstract

TUESDAY, NOVEMBER 15

Symposia—8:00 a.m.
183. New Approaches to Understanding Dendrites
Chaired by: D. JOHNSTON | 427
184. The Amygdala: From Circuits and Synapses to Emotional Memory
Chaired by: J. LEDOUX | 427

Special Lecture—11:15 a.m.
185. Membrane Receptors and Ion Channels: Electron Crystallographic Studies of Their Design and Action
N. UNWIN | No Abstract
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**THEME E: ENDOCRINE AND AUTONOMIC REGULATION**

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**THEME H: OTHER SYSTEMS OF THE CNS**

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**4. Genes With Triplet Repeats in Neuropsychiatric Illness**
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- History of neuroscience
- Teaching of neuroscience: computer programs
- Teaching of neuroscience: curriculum development
- Teaching of neuroscience: laboratory courses and exercises
University of Toronto, Ontario and Harvard Medical School, Boston.

Phospholipase A₂ (PLA₂), the enzymes which catalyze the hydrolysis of the sn-2 fatty acid residue esterified to a variety of phospholipid species, were studied in preparations of autopsied human cerebral cortex. A greater amount of PLA₂ activity was found in the membrane (particulate) fractions when compared with the cytosolic fraction. The particulate and cytosolic enzymes possessed similar characteristics, including having a pH optimum of 8.5, and being maximally active in the presence of millimolar concentrations of calcium ions. The particulate enzyme could be solubilized by IM potassium chloride, and was capable of hydrolyzing phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS), with a preference being displayed for PS and PE over PC. However the enzyme displayed only a small preference for arachidonic over linoleic acid residues at the sn-2 position of PE. When the solubilized particulate enzyme was subjected to gel filtration chromatography, PLA₂ activity eluted as a single peak of relative molecular mass greater than 67kDa. PLA₂ activity was diethiothreitol insensitive, but was inhibited by brief heat treatment, bromophenacyl bromide, AAOCl₂ (the tri-fluoroketone derivative of arachidonic acid) and γ-linoleyl amide. Thus, while the human brain enzyme displays many of the characteristics of low molecular weight platelet PLA₂, it differs in several key features, suggesting that human brain may contain a novel form(s) of PLA₂.

DOCA-INDUCED CHANGES IN SEPTO-PREOPTIC NEURON SENSITIVITY TO ANGIOTENSIN II AND LEUKOCYTE-ENRICHED COORTICOTROPIN-RELEASING HORMONE AND ACTIVATION OF THE COORTICOTROPIN-RELEASING HORMONE (CRF) RECEPTORS.

In the studied area, immunoperoxidase application of all induced activation on 17 (23.3%) and inhibition on 4 (5.5%) of the spontaneous activity of the 73 neurons tested for this peptide in the control animals. In the DOCA prereated rats, all induced activation on 31 (35.6%) and inhibition on 7 (8%) of the spontaneous activity of the 87 neurons tested. These numbers give a ratio of excitatory/Inhibitory responses to All of 81/19 for control and of 82/18 for DOCA. Immunoelectron application of Losartan by itself in this same forebrain region induced activation on 6 (13%) and inhibition on 6 (13%) of the spontaneous activity of the 46 neurons tested in the control animals. In the DOCA group, Losartan induced activation on 14 (26.9%) and inhibition on 1 (1.9%) of the spontaneous activity of the 52 neurons tested, giving an excitatory/inhibitory ratio of 50/50 for control and of 97/0 for DOCA.

These results show that All can be excitatory or inhibitory on neurons of the same region. The number of responses to All is enhanced by DOCA pretreatment but the shift is in excitatory/inhibitory ratio for Losartan, but not for All suggests that several types of receptors are involved in these DOCA-induced modifications.

(Submitted by MH 43787 & Eilas C*)

Lab of Molecular Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 0651.

Corticotropin-releasing factor (CRF) is involved in regulation of the pituitary-adrenal axis as well as the HNS response to stress. The CRF receptor is positively coupled to adenylyl cyclase, however the mechanisms which control CRF receptor expression are poorly understood. In this study we used an immunolocalized locus coeruleus-like (LC-like) cell line, previously shown to contain CRF receptors, as a model system to study regulation of CRF receptor coupling to adenylyl cyclase, ligand binding, and mRNA levels.

CRF treatment resulted in a rapid, time-dependent down-regulation of CRF-stimulated adenylyl cyclase and CRF receptor ligand binding, which was maximal after 20 min, and was observed for up to 2-4 hrs. However, after 20 hrs of CRF treatment there was a further decrease of approximately 20-30 %. Incubation of LC cells also decreased levels of CRF receptor mRNA but with a different time-course: agonist incubation decreased levels of receptor mRNA by 30 % after 4 hrs and by 60 % after 24 hrs. The results suggest that agonist regulation of receptor expression is mediated by several processes. The rapid, probably mediated by receptor sequestration and internalisation as described for adrenergic receptors, and decreased levels of receptor mRNA probably contributes to the more long-term decrease observed after 24 hrs of treatment.

ADRENERGIC MODULATION OF UP- AND DOWN-REGULATION I

ADRENERGIC REGULATION OF β-ADRENERGIC RECEPTOR AND IBERINA IN GLIOBLASTOMA CELLS. J. Rydel, E. Fitzpatrick and R.S. Duman.
Department of Molecular and Developmental Biology, University of California, Los Angeles, CA 90095.

We have shown previously that exposure of C6 glioma cells to isoproterenol decreases the transcription rate of the B₁ and B₂ adrenergic receptor. This decrease in transcription is sensitive to inhibition of protein synthesis suggesting that isoproterenol induces a transcriptional repressor. A potential mediator of the repression is ICER, a member of the CREM (CRE modulator) family of transcription factors, which is induced by activation of the cAMP system, can bind to the CRE (cAMP responsive element), and antagonizes the stimulatory effects of CREB (CRE binding protein) (Stehle et al., Nature '93). Since CRE is present in both B₁R and B₂R promoters, the effects of isoproterenol on ICER regulation in C6 glioma cells were investigated. RNase protection assays reveal that exposure of C6 glioma cells to isoproterenol or forskolin for 1 hour induces levels of ICER, as well as CREM, mRNA approximately 10-fold. ICER and CREM mRNA levels return to control levels following 24 hours of exposure to agonist. The induction of ICER RNA levels by isoproterenol is not blocked by inhibition of protein synthesis. Moreover, ICER mRNA levels are more highly induced in the presence of protein synthesis inhibitors, consistent with reports that ICER can inhibit its own production. Electrophoretic mobility shift assays show that isoproterenol and forskolin induce at least two CRE binding complexes, both of which migrate faster than the CREB containing complex. Supershift assays with a CREB antibody demonstrate that these complexes do not contain CREB and thus may contain ICER. Additional studies will be performed to further characterize the regulation of ICER and to investigate its potential role in the repression of B₁R and B₂R gene transcription.

CHRONIC MORPHINE TREATMENT DECREASES MELANOENIN-INDUCED 4-RECEPTOR mRNA EXPRESSION IN RAT FRONTAL CORTEX. J.D. Albarracín*, E.J. Neistler and R.S. Duman.
Neuroscience Program, Laboratory of Molecular Psychiatry, Department of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06509.

Previously we described the cloning and characterization of a rat melatonin opioid receptor (MC₄-R) (M. Neurosci. 634.9). This receptor is 996b in length and is 95% identical to the amino acid sequence of the published human MC₄-R. In order to study the regulation of MC₄-R by psychotropic drugs, we have investigated the possible effects of morphine addiction on melatonin receptors. Rats were administered 75mg morphine pellets (s.c.) once daily for 5 days, and on the sixth day several brain regions from morphine- and sham-treated animals were dissected. Using an MC₄-R specific riboprobe in a RNase protection assay, we found that MC₄-R mRNA levels in the frontal cortex were consistently down-regulated in morphine-treated animals. To determine whether this effect was specific to MC₄-R, the RNase protection assay was repeated using a riboprobe for MOR-C₃. Down-regulation in the frontal cortex was not observed with a tendency for up-regulation was noted. The effect of morphine treatment on melatonin receptor mRNA will be examined in the other dissected brain regions by the RNase protection assay, and in situ hybridization will be used to localize more discrete regions of receptor regulation. Regulation of MC₄-R expression could contribute to the neurochemical adaptations underlying the long-term actions of opiates in the brain.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
384.5 EFFECT OF SEROTONERGIC DRUGS ON THE UP-REGULATION OF DOPAMINE D2 RECEPTOR EXPRESSION IN HYPOTHALAMUS.

Dept. of Psychiatry, Hokkaido Univ. Med. School, Sapporo 060, Japan.

We examined the modifying effect of serotonin(5-HT) on haloperidol-induced up-regulation of dopamine(D2) receptor in order to elucidate the pharmacological characteristics of antipsychotic drugs on dopaminergic neurons in the hypothalamus of rats with haloperidol administration. Haloperidol (HPD, 0.1-0.5mg/kg, i.p., 3weeks) increased the number of D2 receptors in the striatum, while no increase was observed by that with clozapine (1mg/kg) and OROS-222 (0.25mg/kg), typical antipsychotic drug and its candidate which have high affinity at S-HT2 receptor sites with lower affinity at D2 sites. Chronic treatment with MK212, a nonselective 5-HT agonist and with clonidine, a 5-HT receptor inhibitor (10mg/kg), both of which had no effect on the number of D2 receptor sites by themselves, potentiated the up-regulation of D2 receptor sites when coadministered with HPD (0.5mg/kg). Coadministration of ritanserin(RIT), a 5-HT2/5-HT1c antagonist (1mg/kg), with HPD (0.5mg/kg), had no influence on the HPD-induced increase of D2 receptor sites, but that with smaller dose of HPD(0.1mg/kg) attenuated the D2 up-regulation. These results suggest that serotonergic activity may have a complex modulatory influence on the up-regulation of D2 receptor sites induced by HPD. We are now examining the effect of 8-OD-PAT, a selective 5-HT1A agonist (0.1mg/kg), on the HPD-induced D2 up-regulation.

384.6 EFFECT OF PRENATAL DIAZEPAM TREATMENT ON THE DEVELOPMENT OF DOPAMINE D2 RECEPTORS IN RAT BRAIN.

F. Park, J. L. Long, B. Duration, and L. Svendsen
Douglas Hospital Research Center, Departments of Psychiatry and Neurology, McGill University, Montreal, Que (Canada), H3H 1R3.

Studies on the postnatal development of dopamine receptors have shown a maturation of the receptors between the second and the third postnatal weeks. It has been suggested that the presence or absence of dopamine during prenatal or early postnatal periods may affect subsequent development of dopamine D-1 and D-2 receptors. In the present study we investigated the effects of a dopamine D-2 agonist (quipazine) and of an antagonist (raclopride) on the density and mRNA levels of dopamine D-2 receptors. Quipazine (1mg/kg, s.c.) and raclopride (0.5mg/kg, s.c.) were administered daily from gestation day 10-11 till birth of the pups. Pups were sacrificed at different postnatal ages and their brains were removed and sectioned for anatomical studies. [3H]Sparadone and [3H]raclopride, used in receptor binding studies and in situs hybridisation, respectively. In accordance with previous results, the maximum density of the D-2 receptor and mRNA levels were significantly elevated at postnatal day 28 in rats prenatally treated with quipazine. No significant changes in D-2 mRNA levels were evident in the raclopride treated rats of the same age group. These results suggest that prenatal stimulation of dopamine D-2 (and/or D-3 and D-4 that have high affinity for quipazine) receptors has long-term consequences for postnatal dopamine receptor development. (Supported by the Medical Research Council of Canada.)

384.7 ASTROGLIAL OXYTOCIN RECEPTOR DOWN-REGULATION: MODULATION BY PROTEIN(S) KINASE(S) C. D. Di Scala-Gioeob, Ch. Kelche* and M. Th. Strosser, Institut de Physiologie, URA 1466, 21 rue René-Descartes, 67000 Strasbourg, France.

Specific oxytocin (OT) receptors have been previously characterized on hypothalamic cultured astrocytes and intracellular Ca+2 oscillations by Ca+2 imaging. OT-agonist has been demonstrated. The involvement of phospho-inositol hydrolysis which generates IP3 and diacylglycerol in the activation of protein kinases C (PKC), could be hypothesized. As stimulation of PKC appears to be an important event in transduction mechanisms and receptor downregulation, the role of PKC in olfactory hypothalamic cultured astrocytes. Binding studies were performed on cells pretreated with a phorbol ester (phorbol-12,13-acetate, 500nM) for 24 hours. OT receptor binding (10-7M) alone was without effect on ligand binding whereas simultaneous addition of OT and PMA significantly decreased the binding and this effect was reversed by staurosporin. PMA long-term treatment is thought to desensitize PKC receptors in our system. 18 hours treatment with PMA or OT alone decreased ligand binding and simultaneous application of the drugs potentiated this effect which could not be reversed by staurosporin. In conclusion, the first step in agonist- and phorbol ester-mediated ligand binding inhibition, depends on PRC activation whereas down-regulation induced by long-term treatment is independent from PKC.

384.8 OXYTOCIN BINDING IN HIPPOCAMPUS, VMH AND AMYGDALA: EFFECTS OF STRESS AND GLUCOCORTICOIDS. L. Liperario*, E. A. Young, M. Hsiri, University of Michigan, Ann Arbor, MI, 48104

Neuroanatomical studies reveal that central oxytocin receptors are concentrated in major regions of the limbic system: hippocampus, olfactory nucleus, amygdala, BNST and hypothalamus. We had previously shown that oxytocin receptor binding in hippocampus is regulated by the level of circulating glucocorticoid hormones, using adrenalectomy and corticosterone replacement model. In the current study we examined the effects of non-habituating stress and high dose corticosterone implants on oxytocin binding in non-adrenalectomized animals. Seventeen Sprague-Dawley male rats (250 g) were divided into 3 groups: Controls, Stress and Corticosterone (DO-2) implants (6 males and 5 animals respectively). Animals were sacrificed 1 week after the implantation of two 100mg 100% corticosterone pellets, or 24 hr. after the seventh day of exposure to non-habituating stress (swim; cold room; restrain and ether anesthesia - two stressors/day). Oxytocin receptor binding autoradiography results suggest that non-habituating stress significantly increased oxytocin receptor binding in Amygdala (p<0.05) with trend to increase receptor binding in other areas examined (VMH, Dorsal hippocampus and Ventral hippocampus). High dose corticosterone implants increased oxytocin receptor binding in Dorsal hippocampus and showed trend in Ventral hippocampus. The results of this study further support our early findings regarding the effects of glucocorticoids on oxytocin binding. They provide first preliminary evidence for the effects of non-habituating stress on central oxytocin binding and suggest that glucocorticoids play a role in modulating this effect in hippocampus.

384.9 OLIGO/OXYNUCLEOTIDES TO THE CLONED DELTA OPIATE RECEPTOR, DOR-1:UPTAKE, STABILITY AND REGULATION OF RECEPTOR GENE EXPRESSION. K. M. Standifer*, C-C. Chien, E. J. Pan, and G-W. Fasolka, The Center for Neuro-Oncology, Department of Neurology, Memorial Sloan-Kettering Cancer Center, and 2Dept. Pharmacology, Cornell University Medical College New York, NY 10021 and 3National Taiwan University Hospital, Taipei, Taiwan.

Previously, we reported that phosphodiester antisense oligodeoxynucleotides (ODNs) directed towards various domains of the coding region of the cloned mouse delta receptor, DOR-1, reduced delta receptor binding in vivo and in vitro. Additionally, an antisense directed toward the amino terminus of DOR-1 (Antisense A) blocked DPDPE and deltorphin II-mediated spinal analgesia with no effect on mu- or kappa-mediated spinal analgesia. Subsequently, Porreca demonstrated that the same antisense also blocked supraspinal deltorphin II-mediated analgesia but not that of DPDPE. In this study we examined the ability of Antisense A to enter NG108-15 neuroblastoma cells and mouse spinal cord, and its stability therein. Radiolabeled Antisense A (250 nM) was taken up into cells in a time-dependent fashion, reaching a plateau after 4 hours that extended to several days. Solution hybridization assays using a riboprobe transcribed from the coding sequence of DOR-1 were used to measure levels of spinal cord mRNA after antisense A treatment. The decrease in spinal cord mRNA levels were consistent with loss of binding levels.

384.10 DIFFERENTIAL EFFECT OF LONG-TERM ANTIDEPRESSANT TREATMENT ON 5-HT4 RECEPTOR MEDIATED HYPERTHERMIA IN FAWN-HOODED RATS. Charanjit S. Aulakh, Pascale Mazzola-Pomietto, Anne M. Andrews*, and Dennis L. Murphy, Lab. of Clinical Pharmacology, National Institute of Mental Health, Bethesda, MD 20892.

We have recently demonstrated that hyperthermia induced by 1,2,5-dimethoxy-4-isophenyl-2-amino propanol (DOI) and 5-chlorophenylpiperazine (m-CPP) is mediated by selective stimulation of 5-HT4 and 5-HT2 receptors, respectively (Aulakh et al., 1993). Furthermore, hyperthermia induced by either DOI (Aulakh et al., in press) or m-CPP (Mazzola-Pomietto et al., 1993) was found to be significantly less in the Fawn-Hooded (FH) rat strain that has suggested to represent a genetic model of depression) relative to Wistar rats. In the present study, we studied the effects of long-term antidepressant treatment on DOI (2.5 mg/kg) and m-CPP (2.5 mg/kg)-induced hyperthermia in male FH rats. Long-term (21 days) treatment with the triyclic antidepressants, imipramine or clomipramine (each 5 mg/kg, s.c.) did not attenuate DOI-induced hyperthermia, while m-CPP-induced hyperthermia was accentuated. On the other hand, long-term (21 days) treatment with the monoamine oxidase-A inhibiting antidepressant, zimeldine (26 mg/kg) did not modify m-CPP-induced hyperthermia, but significantly attenuated DOI-induced hyperthermia. These findings demonstrate a differential effect of long-term antidepressant treatment on 5-HT4 receptor-mediated hyperthermia in a genetic animal model of depression.
384.11

During the last decade, several non-benzodiazepine drugs that for the 5-HT1A receptor subtype, including buspirone and gepirone, have been shown to be effective in the treatment of anxiety. Lepotrin is a new piperidino-piperazine that reduces behavioral responses to averse situations in animal models of anxiety and that shows high selectivity and specificity for the 5-HT1A receptor. On the other hand, different lines of pharmacological evidence have led to the hypothesis that the locus coeruleus (LC), the largest noradrenergic nucleus of the rat brain, is a mediator of anxiety and its behavioral manifestations. Moreover, in vivo experiments have shown that serotonin can attenuate LC activity, and serotonin-containing terminals have been described in this brain area. The current study examined the possibility that chronic lepotrin might alter levels of tyrosine hydroxylase (TH) in the LC. Rats were administered lepotrin for 14 days (15 mg/kg), and on day 15 levels of TH immunoreactivity were quantified by immunoblotting. Chronic lepotrin treatment decreased levels of TH immunoreactivity in the LC by 40%. Our results suggest a relation-ship between lepotrin and the noradrenergic system that could be linked to its mechanism of action.

384.13
MODULATION OF GLUCOSE RESISTANCE MEDIANATED CHLORIDE RESPONSES BY PROTEIN KINASE C. Y. Gu* and L. M. Huganir* Marine Biomedical Institute* and Department of Physiology and Biophysics†,† University of Texas Medical Branch, Galveston, Texas 77555-0483.

Glucose is known to inhibit the sugar-synaptic transmission in the spinal and medullary dorsal horns. We have shown previously that protein kinase A potentiates glucose-activated Cl- conductance by increasing the probability of opening of GluCl channels (Gu et al. 1994). To determine whether the second messenger also modulate the glucose responses, we studied the effects of protein kinase C (PKC) on the glucose-activated Cl- conductance in isolated trigeminal neurons. The currents were recorded using the whole cell patch-clamp recording technique and were calibrated with the recorded cells with a fast perfusion method. The protein kinase C and protein kinase inhibitor (PKI) were applied intracellularly through a plastic tube inserted into the patch pipette. PKC was added to the glucose-induced Cl- currents up to 2.5 fold. This enhancing effect of PKC was blocked when PKC (19-31), a phospho-analogue competing for the substrate recognition site of PKC, was introduced into the cells. PKC did not change the affinity of glucose to its receptor. The apparent dissociation constant values were 0.4-6mM and 25.8mM in PKC.

To determine the mechanism of PKC action on glucose-activated Cl- channels, we examined the current-voltage relations of the currents in control and in PKC. PKC changed neither the kinetics nor the voltage dependence of the Glucose responses. The reversal potentials of the glucose-activated Cl- currents remained unchanged after PKC treatment. The effects of PKC on the channel conductance and on the probability of channel openings are currently under investigation. (The work is supported by NIH grants NS03045, NS23061 and NS1255).

384.15

During embryonic development in vivo ACh sensitivity of chick sympathetic neurons increases. Concomitant with these changes we find an elevated expression of α2, α5, α6 (Deva et al '94). Since both pre- and postsynaptic contacts are established during this time, it is difficult to dissociate which of these interactions regulates nAChR expression.

To separate the role of pre- from postsynaptic influences on nAChRs we examined sympathetic neurons in vitro alone or in the presence of either the pre- or the postsynaptic partners. The level of nAChR subunit expression was measured in individual cells with single channel recordings. Single channel recordings of innervated neurons and neurons contacting target were performed as well, for comparison of changes in nAChR expression vs. nAChR-channel properties. We then systematically evaluated the subunit expression levels, we developed a PCR essay. In contrast to the upregulation of α3, α5 and α7 with both input and target contact, target contact alone decreases the expression of α3 while increasing that of α7. Also we have seen that changes in nAChR subunit gene expression with target contact, all of the nAChR-channels expressed by neurons prior to innervation or target contact are suppressed and a single class of large conductance (~60-70pS) is expressed. Since innervation by preganglionic neurones upregulates the expression of α3 subunits and the number of channels expressed by sympathetic neurons, these experiments suggest that input and target contact collaborate in the regulation of the number and properties of nAChRs.

384.12

Previously, we reported that chronic cocaine administration induced changes in brain benzodiazepine (BZD) receptor binding in brain areas associated with the mesocorticolimbic system and possibly involved in the development of behavioral sensitization. Adult male Sprague Dawley rats were injected once daily with cocaine (14 mg/kg, IP) or saline for 2, 4, or 8 weeks, and sacrificed for 24 hr, 14 days, or 28 days post-treatment to determine the effects of withdrawal from BZD receptor binding. BZD receptors were visualized autoradiographically using [3H]Ro 15-1788. Cocaine induced minimal changes in BZD receptors 24 hrs post-cocaine, yet these rats exhibited significant increases in stereotypy after 2 weeks of cocaine, suggesting that cocaine-induced alterations in BZD receptors were not essential for the development of behavioral sensitization. BZD receptors were increased in the rostral nucleus accumbens, and in cortical areas 14 days post-cocaine, but returned to control values 28 days post-cocaine. Cocaine-induced increases in BZD receptors in these rats were related to the dose of drug, the length of exposure, and the time point of drug withdrawal. In addition, the effects of cocaine on BZD receptors may differ between Mista and Sprague Dawley strains of rats. [Supported by NIH grant DA04293]

384.14

[3H]DTG (1,3-di-o-toylguanidine) binding to the homologous rate of rat cerebral cortex was competitively inhibited by a variety of neurotoxic drugs such as pentazocine, haloperidol, clomipramine and imipramine, which increased the Ki value with no changes in the Bmax value of the [3H]DTG defined sigma binding site as well. In contrast, a classical anti-depressant desipramine reduced the Bmax of the [3H]DTG binding in a concentration-dependent fashion without effects on its Ki. This non-competitive inhibition is unlikely to be related to the blocking action of desipramine at the N- methyl-D-aspartate receptor because selective competitive (CGS9775) and non-competitive (MK-801) antagonists of the excitatory amino acid receptor failed to mimic the effects of desipramine. Furthermore, potent antagonists of the beta-adrenergic receptor, (-)-propranolol and (-)-alpenor- nalol also caused a non-competitive inhibition of the [3H] DG binding. Together with the potent blocking action of desipramine at norpinephrine uptake, the present results suggest that noradrenergic systems might allosterically interact with the sigma-like site or that the sigma macromolecule could have certain allosteric regulation sites which would be affected by desipramine, propranolol and alpenoronal.

384.16

The hippocampus has high levels of both NMDA and non-NMDA receptors. Previous studies have shown the CA1 region of the hippocampus to be particularly sensitive to ischemia-induced neuronal damage. Based on the observation that high-density DHTP phosphate (DHTP) replacement on [125I]MK801 binding in the dorsal hippocampus. Adult Sprague-Dawley male rats were castrated and implanted subcutaneously with either a 25 cmSilastic capsule that contained DHTP (n=3) or an empty capsule (n=3). Control rats (n=8) were left intact. Animals were sacrificed 21 days after castration. In control rats [125I]MK801 binding was highest in stratum oriens and radiatum of CA1 as well as in the molecular layer of the dentate gyrus. In castrated rats [125I]MK801 binding was significantly increased in the pyramidal cell layer, stratum oriens and radiatum of CA1 compared to intact controls. This increase in [125I]MK801 binding was prevented by treatment of castrated rats with DHTP. [125I]MK801 binding in the hippocampus of castrated-DHTP treated rats did not differ significantly from intact rats in any region of the hippocampus. These data suggest that androgen receptor stimulation may influence excitatory amino acid neurotransmission within the hippocampus. (NSF BNS-910206, NIH DA06687, DE06682, DC01086, AA8086)
MODULATION OF NMDA CURRENT BY FATTY ACIDS IN MOUSE CORTICAL NEURONS. S.P. Yip*, L.L. Dungan and D.W. Choel, Dept. of Neurology and Center for Neuroscience and Zyklus System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

We previously showed that fatty acid-induced changes in neuronal membrane fluidity were modulated with alterations in NMDA currents (C∗) accumulation and death (Dungan et al., Soc. Neurosci. Abst. 18:756, 1992). Modulation of NMDA current (I_{NMDA}) by fatty acids was studied in cultured mouse cortical neurons using whole-cell voltage-clamp at -70 mV. Each fatty acid was applied at 50 μM together with 50 μM bovine serum albumin. I_{NMDA} in untreated cells was 1.9 ± 0.11 nA (SEM, n=20), and showed no obvious run-down (ATP was included in the recording pipette). (18:1, Δ9) block (50 μM) reversed NMDA membrane fluidity (50 μM), mixed excitatory amino acids (100 μM), cyclooxygenase inhibitor ibuprofen (50 μM), mixed excitatory ion (20 μM), or the kinase inhibitor, staurosporine (1 μM) failed to prevent arachidonic acid-induced potentiation. 

Dicycliydro (50 μM), which decreases membrane fluidity blocked 15% of I_{NMDA}. We are attracted to the idea that degree of unsaturation or impact on membrane fluidity may be important determinants of the effect of fatty acids on NMDA current. Fatty acid-induced modulation of NMDA receptor current may influence the state of this receptor in physiological or disease states. Supported by NIH grants NS 30373 (DWC) and AG05959-01A1 (LD).

844.19


The insulin-like growth factors (IGF-I and IGF-II) and insulin are localized in distinct brain regions and their receptor functions are mediated by specific receptors. High concentrations of binding sites for these growth factors are discretely distributed throughout the brain, including the hippocampal formation. Functionally, IGFs and insulin in addition to their growth and trophic effects, are considered to play important roles in normal cell functions as well as in response to pharmacological or surgical manipulations. Previously, we have shown that systemic injection of insulin-like growth factors (IGF) to adult rats altered IGF binding sites in discrete layers of the hippocampal formation (Shrivastava et al., Soc. Neurosci. Abst., 1991). Since IGFs and insulin play important regulatory roles during critical periods of rat life, the present study was designed to evaluate the response of IGF and insulin binding sites at different ages following systemic injection of IGF-II to newborn rats. KA was injected to post-natal day 1 pups (5mg/kg, i.p.) and 1.25-IGF, II, 1-IGF-II and 1-IGF-II binding sites were studied at different time periods (7, 14, 21, 28 and 35 days) using quantitative autoradiography. In the neonatal hippocampus, 1-IGF-I binding sites are concentrated primarily in the dentate gyrus (DG) and the CA2-CA3 sub-fields whereas 1-IGF-II binding sites are discretely localized to the pyramidal layer and the granular layer of the DG. insulin binding sites are mostly present in the molecular layer of the DG and the CA1 sub-field. Following KA injection, the level of IGF-I binding sites was increased on days 14 and 21 before returning to normal values at later times. In contrast, IGF-II and insulin binding sites decreased from day 7 to 14 in post-natally treated rats. Taken together, these results provide further evidence for the distinct existence of IGF-I, IGF-II and insulin binding sites in the rat hippocampal formation. The transient increase in IGF-I binding sites at days 14 and 21 may relate to cell proliferation caused by the KA treatment. (Supported by MRCC and The Alzheimer Society of Canada).
**384.23**

**DOPAMINE (DA) RECEPTOR-C-FOS COUPLING IN THE DA DEAFFERENTED AMYGDALOID COMPLEX**

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The autoradiographic localization of DA receptors in the rat amygdaloid complex indicated a topographic, nonoverlapping distribution of the D1 and D2 receptor subtypes in its component nuclei and subnuclei zones (Byrne 1986, 1992). The functional compartmentation of amygdaloid DA receptors and their comparison to striatal DA receptors was further assessed by the effect of a unilateral 6-OHDA lesion of the MFB on the expression of fos-D1 in central (Ce) and basolateral (BL) amygdaloid nuclei to D1 or D2, D1 or D2 receptor activation. The D1 agonist SKF 38393 (3 mg/kg sc) produced a marked increase in fos-LI in the medial and dorsolateral caudate nucleus; the effect of SKF 38393 was enhanced greatly by the coadministration of quipiprole (0.3 mg/kg sc). Quipiprole did not induce fos-LI when administered alone. Though quantitatively smaller, similar effects of D1, D2 and D3 receptor activation were observed in the core and shell of the nucleus accumbens and olfactory tubercle ipsilateral to the side of the MFB lesion. A strikingly different pattern of effects of activation of DA receptor subtypes was observed in the DA deafferented amygdaloid complex. Specifically, the administration of apomorphine (0.3 mg/kg), SKF 38393, or the combination of quipiprole and SKF 38393 increased fos-LI in BL, though no differences in drug effects were noted between the ipsilateral and contralateral (intact) BL. These preliminary results suggest that D1 receptors in BL induce fos expression by an intracellular pathway that is not upregulated in response to deafferentation. The apparent lack of effect of D2 receptor activation on the D1 response is consistent with the negligible localization of D2 receptors in BL. In contrast, the increase in fos-LI in Ce resulting from D1/D2 receptor activation was greater in the lesioned versus intact Ce.

**384.24**

**Down-regulation of neurotransmitter receptors and up-regulation of neurofilament phosphorylation in the lateral geniculate nucleus of the adult cats after visual deafferentation.**

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Several progressive neurodegenerative disorders such as Parkinson's disease and Alzheimer disease have been associated with abnormal neurotransmitter receptor expression and neurofilament distribution. To test the hypothesis that the redistribution and regulation of neurotransmitter receptors and cytoskeleton protein phosphorylation may trigger early neurodegenerative process induced by input deprivation, we investigated the response of several signal transduction and cytoskeletal markers to removal of retinal input in the lateral geniculate nucleus. Nine neurotransmitter receptors, including α1 and β-adrenergic receptors, muscarinic acetylcholine receptors, as well as L-type calcium channel, protein kinase C and phosphorylated neurofilaments were examined autoradiographically and immunocytochemically in the lateral geniculate nucleus (LGN) of eight adult cats at various times after monocular visual deafferentation. While α1 and β-adrenergic receptors, and muscarinic acetylcholine receptors were down-regulated in the deafferented layers of the LGN as early as 8 days after enucleation, phosphorylated neurofilaments were increased in the same layers as early as 4 days after enucleation. These results may suggest that the early changes in density of adrenergic and muscarinic acetylcholine receptors and phosphorylated neurofilaments are involved in the neurodegenerative process induced by input restriction, and may be useful as markers for neurodegeneration.

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**HYPOTHALAMIC-PITUITARY-ANDRENAL AXIS REGULATION: STRESS STUDIES**

**385.1**

**STRESS-INDUCED TRANSCRIPTIONAL ACTIVATION OF THE CORTICO-TROPIN-RELEASE INHIBITORY FACTOR GENES PRECEDES IMMEDIATE-EARLY GENES RESPONSES IN THE PARAVASCULAR NUCLEUS.**

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Activation of nuclear protein kinase C (PKC) is an integral element in the signaling pathway coupling the depolarization of the plasma membrane to the transcriptional regulation of genes coding for acetylcholine receptor (AChR) subunits. Since PKC activation is immediately followed by genetic inhibition we assume that a transcription factor is directly targeted by the kinases. The muscle-specific expression of ACR and the presence and functional significance of B boxes in the ACR subunit promoters strongly suggest that myogenic determination factors are involved. Among them myogenin is a good candidate because its activation and inactivation kinetics resemble that of the endogenous transactivator. Thrombin 87 in mouse myogenin has been shown to be a target for PKC, but since it is part of the DNA binding domain it may not be accessible to the kinase in the DNA-bound factor. To determine the phosphorylation site responsible for the rapid inactivation by PKC, we have systematically replaced all eight potential PKC targets in the chicken myogenin molecule and analyzed the PKC sensitivity of the mutant factors. Results of this analysis will be presented.

**385.2**

**HIPPOCAMPAL CHOLINERGIC BLOCKADE ELEVATES STRESS-INDUCED CORTICOSTERONE (CORT) SECRETION.**

S. Bhattacharya, J.W. Snyder and M.J. Myers.


The hippocampal formation (HPC) regulates stress-induced hypothalamic-pituitary-adrenal (HPA) activity. Lesions of the HPC result in hyperserection of ACTH and CORT in response to a variety of stressors, suggesting that the HPC plays an inhibitory role in the regulation of HPA activity. Septal cholinergic projections innervate all fields and subfields of the HPC and increase HPA excitability. These stressor-induced inputs are thought to regulate sensory processing of stressful environmental events, as suggested by studies on HPC electrophysiology. We investigated whether HPC cholinergic systems might underlie HPC control of HPA function. Adult rats were implanted bilaterally with cannulas aimed at the molecular layer of the dentate gyrus. Scopolamine HCl (SCOP; 10 μg/μl; 5 μl total) was administered into the HPC 20 min prior to onset of restraint stress. Plasma CORT levels were measured immediately following and up to 2 hours following termination of restraint. Rats administered SCOP (n=6) hypersecreted CORT at 0 min and showed elevated levels 2 hours following termination of restraint, relative to vehicle injected rats (n=6). These data suggest that HPC cholinergic systems actively inhibit HPA activity. Thus, compromising HPC cholinergic inputs may interfere with sensory processing and alter the ability of the HPC to exert negative-feedback control of HPA activity in response to stressful stimuli. (Supported by MRCC and FRSQ)
A CIRCULANT NITRIC OXIDE SIGNALING MECHANISM MEDIATES STRESS-INDUCED CORTICOSTERONE RELEASE. Y. Blackwood1,2, D. O’Donnell1,2, B. Aron1,2, C. Consden1,2, M. D. McGinnis1,2, Canadian, McGill University, Montreal, Quebec, Canada.

Nitrile oxide synthase (NOS) containing neurons have been localized in the hypothalamic-neurointermediate lobe (PNV) and, in vitro studies suggest that nitric oxide (NO) is involved in the release of corticotropin releasing hormone (CRH) from PNV neurons. Because CRH can play a role in the regulation of pituitary-adrenocorticotrophin (ACTH) and corticosterone (CORT) secretion from the adrenal cortex, we investigated the effects of blockers of NO synthesis on the corticosterone response of rats. Immobilization stress for 60 min caused a significant increase in cortisol and ACTH response of anterior pituitary cells. Pretreatment with the NOS blocker N-nitro-L-arginine methyl ester (LNAME, 10 mg/kg, p.o.) did not block the increase in CORT production and the adrenal cortex. This suggests that NO may be involved in the regulation of the stress-induced increase in cortisol and glucocorticoid levels following immobilization stress.

385.3

385.4

INVOLVEMENT OF C-FOS IN DEVELOPMENTAL REGULATION OF CRH GENE EXPRESSION BY COLD-STRESS. Z-J. Li, P.S. Gott, and T.Z. Baran. Neurology, Children’s Hospital Los Angeles and University of Southern California, Los Angeles, CA 90032. C-fos expression is induced by a variety of stresses, including stress, to regulate transcription of target genes. These genes often contain cyclic AMP-responsive elements (CREs). The corticotropin releasing hormone (CRH) gene promoter contains the c-fos dependent cascade. We postulated that c-fos-mediated induction of CRH gene promoter involved upregulation of c-fos. We successfully showed that cold-stress induction of CRH gene expression is developmentally regulated: CRH mRNA abundance increased by 4.8 fold after cold-stress on postnatal day 7 (PND9) or 16, but not on day 3. In this study, we tested the hypothesis that c-fos induction on PND6 would induce both c-fos and CRH gene expression. Rats were subjected to cold-stress-anesthesia, and implanted unilaterally with a cannula directed to the paraventricular nucleus (PVN) of the hypothalamus. Rats were sacrificed 4 hr after saline or dibutyryl cAMP (db-cAMP, 100 µm) injection via the cannula. Fos-like immunoreactivity (Fos-IR) in the central amygadoid nucleus (ACE) was increased by db-cAMP infusion on PND9. Cold-stress induced hippocampal Fos-IR, with a further increase by db-cAMP in both ages. Fos-IR in ACE was enhanced following db-cAMP infusion only on PND9. An additive effect of cold stress and db-cAMP on plasma corticosterone levels on PND9. These data support a role for immediate early genes and cAMP-stress-induced upregulation of CRH-gene expression in limbic structures.

385.5

DIFFERENTIAL REGULATION OF EGF AND TGFα mRNA IN RAT PITUITARY AND HYPOTHALAMUS INDUCED BY STRESSES. X. Fan, G. T. Magle1, T. J. Collins2, O. D. Little3, and J. M. McEwen4. Department of Anatomy, Neuroendocrinology, University of Texas Medical Branch at Galveston, Galveston, TX 77555.

Evidence has shown that both epidermal growth factor (EGF) and transforming growth factor (TGFα) are present in the median eminence and hypothalamus, and EGF can influence the function of pituitary cells, particularly corticosterone in vivo and in vitro. However, little is known about their exact functional roles in the pituitary and hypothalamus. This was due to the difficulty in detecting EGF and TGFα mRNA in the rat pituitary and hypothalamus. Reverse transcriptase-polymerase chain reaction (RT-PCR) also showed the presence of EGF and TGFα mRNA in those areas and other rat tissues (mesencephalon, liver, kidney, hypothalamus, and testis). No TGFα mRNA was found in the kidney however. The EGF mRNA is upregulated in the pituitary after stress in pregnant rats and rat cold-stress stress (SSS) but not after 30 min after stress (NS) or tape-stress (TS). Older cold stress showed that expression of EGF mRNA was downregulated after 60 min cold stress and then upregulated after 180 min post-stress response. In contrast, EGF mRNA in the hypothalamus is not responsive to either acute stressors or longer periods of cold exposures. No significant change in TGFα mRNA expression was detected in both pituitary and hypothalamic after acute and longer cold stressors. The results suggested that the change in pituitary EGF mRNA in response to stress varies according to the type of stress and may be under the influence of glucocorticoid feedback. Preliminary evidence suggests that the corticosterone may be one of the cells that express EGF mRNA in the pituitary. Our data further support that EGF is a stimulator of HPA axis in primates. These stress-induced changes in EGF mRNA suggest that it may play an important role during stress responses. Supported by NSF # NIH-1917897.

385.6


Learned helpless behavior has been successfully bred in rodents and designated as an animal model of human depression and/or anxiety. Since learned helpless (CHL) animals have an altered response to stress in adulthood, we examined the effects of early stresses (at day 7, 14, and 21) on the hypothalamic-pituitary-adrenal (HPA) axis and the cardiovascular system. The functioning of the HPA axis was monitored through changes in adrenocorticotropic (ACTH) plasma levels in the adult animals after acute stress exposure in adulthood. Because ACTH and PRA are known to be controlled by the HPA axis and the sympathetic nervous system, we studied the effects of early stress-induced stress in adulthood. We measured ACTH and PRA in the sera of CHL and control animals before and after stress (acute stress). In conclusion, CHL animals (adults) that were housed postnatal day 14 had lower plasma levels of ACTH than control, while exhibiting a 9% increase in PRA, again pointing to a reduction in functional cardiovascular system. The most robust effect of MD on the CHL adult animals was apparent after acute stress on postnatal day 14 as opposed to postnatal days 7 and 21. In contrast, there was a step-wise increase in ACTH plasma levels in the congenital non learned helpless (NLH) rats with age of acute presentation of stress. MD. The above results suggest that there are long-term changes in both the HPA axis and the cardiovascular system in CHL animals after acute exposure to a postnatal stressor.

385.7

IN VIVO QUIPANZEE INCREASES GLUCOCORTICOID RECEPTOR EXPRESSION IN THE HIPPOCAMPUS OF NEONATAL PIGS. S. Weyer1, D. O’Donnell1, A. Schoerl1, L. Thibault1,2, and M. J. Masey1,2. 1Dept. Animal Sci., Univ. of Alberta, Edmonton, AB, Canada; 2Dept. Physiol. Sci., Univ. of Guelph, Guelph, ON, Canada.

The glucocorticoid receptor (GR) in the hippocampus is involved in the negative feedback effects of glucocorticoids on stress. The handling stress results in increased GR binding capacity in the hippocampus and frontal cortex and an attenuation of the stress response in rats. The postulated mechanism is via increased expression of the GR (5-HT) turnover in the hippocampus (Mitchell et al., 1990, J. Neurosci. 10745). Little is known about the presence or development of GR in porcine brain. Brain is associated with dramatic costs in the swine industry due to impaired animal growth, reproduction, and meat quality and reducing its impact represents significant savings. In the current study we examined the effect of the 5-HT agonist quipazine on GR levels in specific brain areas using Western blotting with a commercially available GR antibody (Affinity BioReagents). Pigs were injected with 1 mg/kg quipazine dissolved in saline (1 ml/kg) or saline from postnatal day 1 to 14. The animals were sacrificed on day 13, and the hypothalamic, frontal cortex, hypothalamus, and hippocampus were collected. Increased levels of immunoreactive GR were detected in the hippocampus of quipazine versus saline treated pigs. We are currently analyzing the remaining tissues to determine whether the changes in GR expression are confined to the hippocampus and frontal cortex as is the case for neonatally handled rats.

385.8

PRENATAL STRESS-INDUCED CHANGES IN THE ACTIVITY OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS OF ADULT OFFSPRING DEPEND ON MATERNAL CORICOSTERONE. S. Macaor5,6,7,8, A. Barbasz8, F.J. Pineau1, H. Stinson2, J. L. Mor6, W. Lavoie4, M. Lavoie4, Karin5, INSERM U1259, Université de Bordeaux II, 33707 Bordeaux Cedex, France.

The development of the autonomic nervous system during the perinatal period. Prenatal stress can have long-term behavioral effects, such as an increased emotional reactivity and a higher vulnerability to self-administer drugs. Changes in the activity of the hypothalamic-pituitary-adrenal (HPA) axis is a sensitive index of the integrated response of the brain to stress. The HPA axis is a complex system of the brain, and it has been proposed that one of the most critical components of the stress response is the number of central corticosteroid receptors. The mechanisms by which prenatal stress could mediate its long-term effects on the activity of the HPA axis are unknown. To determine if we could influence the development of HPA axis responses by manipulating the activity of the HPA axis of adult (3 months of age) male and female offspring. Repeated restraint during the last week of pregnancy was used as a stressor. In adult male and female offspring of mothers subjected to repeated stress, the HPA axis was increased in maternal corticosterone receptor expression. Such effects of prenatal stress depend on maternal corticosterone-secretion: 1) maternal adrenalscrotal protected the offspring from the effects of prenatal stress by cortisol receptor; 2) injections of corticosterone to the mother had the same effects of prenatal stress in adult offspring. In conclusion, the HPA axis may be regulated by one of the mechanisms by which prenatal stress exercises its long-term effects on the activity of the HPA axis.
385.9 EFFECTS OF STRESS ON CRF AND ITS RECEPTOR GENE EXPRESSION IN THE BRAIN OF SPONTANEOUSLY HYPERACTIVE RATS. Guy, J.-D.; de Matos, A.; Mélis, F.; Parent, J.-P.; Prévost, S. Dept. of Neuroendocrinology, Center for Research in Brain Pathologies, CHUL, Quebec, Canada.

The present study investigated the influence of immobilization stress on corticosterone levels and the expression of CRF and CRF-R (CGRP) mRNAs in rats with spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats. Male rats (13 weeks) were exposed to immobilization stress by being placed in a restrainer for 0, 30, 60, and 180 minutes after the beginning of the immobilization session which lasted 60 min. Brains were cut in 60 μm sections and mounted on poly-l-lysine-coated slides. CRF and CRF-R mRNAs were detected by in situ hybridization using a 35S-labeled riboprobe. CRF-R mRNA was detected in the PVN, GEA, and BST of SHR rats. Immobilization stress induced a significant increase in CRF mRNA in the PVN, GEA, and BST of SHR rats. CRF-R mRNA was observed in both the PVN and BST of SHR rats. The results provide evidence that central CRF system may play different roles in stress in various species, particularly in the SHR and WKY rats.

385.10 IMMUNE CHALLENGE INDUCES CRF RECEPTOR GENE EXPRESSION IN THE RAT PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS. Serge Rivest*, Laboratoire d’endocrinologie moléculaire, Centre de recherche du CHUL, Québec, Canada.

The present study investigated the effect of intraperitoneal (i.p.) administration of the endotoxin lipopolysaccharide (LPS) on the gene expression of CRF and CRF-R (CGRP) mRNAs in the paraventricular nucleus (PVN) of male Sprague-Dawley rats. One, 3, 6, 9, and 12 hrs after a single i.p. injection of either the LPS (250 μg/100 g of BW) or the vehicle, sham rats were decapitated and rapidly perfused with 4% paraformaldehyde. Brain was microtomed and cut from the olfactory bulb to the medulla in 30 μm coronal sections. mRNAs encoding the rat CRF and CRF-R were detected by in situ hybridization histochemistry using a 35S-labeled riboprobe. The Fig. CRF-R cDNA in Blueprints vector (pcRF-R-PAI-3S, 4.3 kT) was kindly provided by Dr. W. Vale (The Salk Institute, La Jolla, CA). A strong basic level of CRF-R mRNA transcripts were observed in several regions of the brain such as the lateral nucleus, the dorsal gray, the hypothalamus, and the septum. In the cortical zone, the density of the zone increased, the endopine nucleus and in various layers of the corpus. A low to moderate signal was also detected in multiple sites including the medial septal nucleus, the nucleus of the diagonal band, the periaqueductal gray, the nucleus prepositus, and the ventral mesolimbic nucleus. While vehicle-treated rats did not display detectable signal of CRF-R mRNA in the paraventricular nucleus (PVN), CRF-R gene expression was highly stimulated in this hypothalamic structure. Indeed, the CRF-R mRNA signal was positive in the dorsal medial parvocellular PVN 3 hrs after LPS injection, strong and maximum at 6 hrs postinjection, and declined 9 and 12 hrs after the treatment. Systemic endotoxin did not appear to modulate the expression of CRF-R gene in other regions suggesting that this type of stress is likely involved in the stress-induced CRF within the paraventricular PVN. The results suggest that CRF may provide the control of the inflammatory response and the release of neurogenic CRF during immune challenge. (Supp. by the FRGS)

385.11 DEVELOPMENTAL ONSET OF HYPOTHALAMO-PITUITARY-ADRENAL AXIS RESPONSIVENESS TO NGF IN RATS. G. Tagliatela-Scafati* and J.P. Perez-Polo. Dept. of HBCG, UTMB Galveston, Texas.

Nerve growth factor (NGF) is involved in the regulation of the hypothalamic-pituitary-adrenal axis (HPAA). Peripherally-injected NGF stimulates the activity of the HPAA, resulting in a long-lasting increase in plasma corticosterone levels, whereas serum NGF levels increase, response to stress and ACTH, and pituitary CRF levels. NGF was demonstrated to have a full stimulatory effect on HPAA activity after 14 days of age, consistently with complete hippocampal maturation, we studied the effect of NGF on HPAA in pups at different ages of postnatal development. NGF was administered to pups at 2 days of age, and the response to stress and ACTH was monitored. NGF did not affect the increase in plasma corticosterone levels in pups of 3, 8, and 11 days of age, while a significant response of the HPAA to NGF was observed in pups at 15 and 22 days of age, as well as in adult rats. The results suggest that NGF administration to pups at different ages can modulate HPAA activity by a non-genomic pathway.


SNS and HPA activity both increase in response to acute restraint stress. Brown adipose tissue (BAT) activity, which promotes nonshivering thermogenesis, is an index of SNS activity. We hypothesized that a hyperalgesic load would separate basal activity in the SNS system and tested whether both stress responses were facilitated due increased tone in SNS. Therefore, rats were implanted with thermistor probes under urethane anesthesia, and were monitored by telemetry. The rats were tested for restraint on d10 while BAT temperature was recorded every 60s. On d5 & 10, restraint did not differ from controls in basal activity of the HPA axis, insulin and glucose. On d5, no significant effect on the response of CRF-R or ACTH to restraint. On d10, there was no difference in ACTH response, but a smaller B response at 30m as well as lower integrated B. Both control and sugar rats increased BAT temperature in response to restraint but sugar rats had a smaller increase than controls. SNS (BAT activity) responds to sugar with chronic elevation of SNS tone similar to HPA responses to chronic stress. In contrast to our initial hypothesis, the chronically sugar-activated SNS hyporesponds to acute restraint in parallel with the HPA hypersponse in ACTH and B secretion. We conclude that a hyperalgesic load to acute stresses leads to increased SNS and HPA responses to acute restraint from high to low amplitude. (supported by DK21872 to M.F. Dulinan and HAHS8-42 to AMS)

385.13 IMMEDIATEly EARLY GENE INDUCTION IN RAT FOREBRAIN GABAERGIC NEURONS IN RESPONSE TO AN ACUTE STRESS. W.E. Cullinan* and S.I. Watson, Mental Health Research Institute, University of Michigan, Ann Arbor MI, 48109-0720.

Recent data have suggested that the hypothalamic paraventricular nucleus (PVN) receives a prominent GABAergic input; this innervation has recently been shown to be involved in part from various hypothalamic nuclei and the bed nucleus of the stria terminalis (BST) (Roland and Sawchenko, 1993; Cullinan et al., 1993). These areas have also been shown to express immediate early genes in response to acute stress. The immediate-early gene expression was investigated to determine whether GABAergic neurons in these regions expressed early genes (c-fos, zif/268) following acute stress. Rats were subjected to 100 forced swim at 37°C, and were sacrificed 30 min. post stress. Stress was found to induce c-fos mRNA, with c-fos mRNA levels peaking at 60 min. and then decreasing to basal levels.

385.14 ADRENALECTOMY DOES NOT ALTER STRESS-INDUCED C-FOS EXPRESSION IN THE RAT HYPOTHALAMIC-PITUITARY-ADRENAL AXIS REGULATION: STRESS STUDIES. 937

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385.15

In vitro studies support a model in which the unoccupied corticosteroid receptor is not located in the cytosol, whereas the hormone-activated receptor is present in the nuclear fraction. We have used the western blot procedure to measure cytosolic and nuclear levels of Type II corticosteroid receptor in hypothalamic and other corticostereoid binding tissues, from cytosol or immunoprecipitates of cytosol and nuclear extracts which were separated by PAGE and transferred to nitrocellulose. Blots were probed with Type II corticosteroid receptor reactive antibodies (CR1057 or Bio-Rad) and immunoreactivity was visualized using an antibody quantitated by a chemiluminescence method (ECL, Amersham). Cytosolic Type II receptor binding level and western blot immunoreactivity were measured in rats from hippocampus or cortex from rats that were sacrificed (in the AM) under 4 hormone conditions: 1) adenal-intact, no stress, 2) 18 h ADX, 3) 5 d ADX, 4) adrenal-intact + 2 h ADX (receptor upregulation), and a 70-90% decrease with acute CORT treatment (receptor activation). In recent studies a large increase in nuclear receptor signal was detected when the western blot in CORT treated animals. These studies indicate that the western blot procedure can be used to directly measure both the cytosolic and nuclear level of corticosteroid receptors in rat brain tissue, and that cytosolic changes with hormone manipulations parallel those seen with receptor binding. (Supported by MH47674, MacArthur Foundation)

385.17
MODULATION OF 5-HT2C RECEPTOR mRNA EXPRESSION IN THE HIPPOCAMPUS DURING THE CIRCADIAN RHYTHM AND FOLLOWING STRESS. M.C. Holmes*, K.L. French and J.R. Steck, Dept. Med., Univ. of Edinburgh, Western General Hosp., Edinburgh, UK. Serotonin has been implicated in the generation and maintenance of circadian rhythms, as well as the modulation of HPA axis activity to certain stressors. 5-HT2-type (2A and/or 2C) receptors in the hippocampus and hypothalamus may mediate both actions. Using in situ hybridization, we examined 5-HT2A and 5-HT2C receptor mRNA expression in mice under conditions of the glucocorticoid diurnal rhythm. At 08.00h (lights on 07.00-19.00h) 5-HT2C receptor mRNA expression in the hippocampus was significantly greater than controls at 20.00h (CA1, 184%; CA2, 198%; ventral CA1 147%). 5-HT2A receptor mRNA expression was unchanged. Receptor gene expression was also determined following acute (6h after laparotomy) and chronic (15 days adjuvant-induced arthritis) stress. 6h after acute stress (20.00h) 5-HT2C receptor mRNA expression was significantly elevated in CA2 neurons (66% greater than controls). Chronic arthritis elevated plasma corticosterone, abolishing the circadian rhythm, and 5-HT2C receptor mRNA expression was significantly decreased in CA1 (55%), CA2 (50%) and ventral CA1 (45%) at 08.00h, giving levels of expression similar to the normal diurnal glucocorticoid peak (20.00h). Again, 5-HT2A receptor gene expression was unaltered. Our previous data showed that glucocorticoid regulates hippocampal 5-HT2C receptor gene expression following adrenalectomy. However, in the hippocampus 5-HT2C receptor mRNA expression following acute stress suggests that other factors (perhaps 5-HT) are also important short-term regulators of expression of this gene.

385.18

Corticosteroids (CORT) are known to mediate stress responses, and circulating plasma corticosterone is increased in the elderly. The purpose of this study was to evaluate the effects of antidepressant (AD) treatment on basal HPA activity in aged rats. We found that basal HPA activity was increased in aged rats, and that AD treatment significantly reduced basal corticosterone levels. The mechanism by which AD treatment reverses basal HPA hyperactivity is not yet understood.

385.2
NEUROTOXIN-STIMULATED HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS FUNCTION IN MICE. M. Carino, B. Giovannetti, R. Hillal and E. Szinro. Neuroendocrine Unit, Department of Neurosciences, IMBB, 1900 La Plata, Argentina.

The aim of the present study was to elucidate whether snake venom (SV; Sigma Chem. Co., V-17259) is able to stimulate the HPA axis activity when administered (i.p. 2000 IU/kg) to both sexes. Mice were killed at 0.5, 1, 2 or 4 h after SV or vehicle (sample time zero) administration. Plasma glucose (G), ACTH and corticosterone (B) concentrations were measured. The results indicated that, SV treatment induced a significant (P<0.05 vs. 0 h) hyperglycemia at all periods studied with maximal values attained at 2 h after treatment, regardless of sex; the neurotoxin-stimulated G release in plasma was significantly (P<0.05) higher in females than in males at 0.5, 1 and 2 h after administration. Neurotoxin injection significantly (P<0.05 vs. 0 h) increased ACTH release in plasma at 2, 4 and 6 h after treatment or in both sexes, 1 h after treatment in animals of both sexes, with 1 h-vale significantly (P<0.05) higher in males than in females; plasma ACTH levels remained elevated throughout the 6 h period, with no sex differences. The data suggest that chronic neurotoxin-stimulated G release enhances glucocorticoid negative feedback sensitivity and reduces basal PMS activity in mice. These results are consistent with previous findings in young adult animals and point to potential therapeutic approaches in the treatment of HPA dysfunction in the aged. Supported by MBCC and NIA AG09488.

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HYPOTHALAMIC-PITUITARY-ADRENAL AXIS REGULATION

DECREASED HIPPOCAMPAL NORADRENALINE DOES NOT AFFECT ELECTRICALLY STIMULATED CORTICOSTEROIDE RELEASE. W.M.C. Daniels, A. Jaffer, Y.A. Russell, S. Davy* and J.J.P. Taliard. Dept. of Phys. Path., Univ. Stellenbosch, Tygerberg Hosp., P.O. Box 1311, Tygerberg, 7505, RSA.

Bipolar electrodes were implanted into the CA1 pyramidal cells of the dorsal hippocampus and the effect of electrical stimulation of these cells on corticosterone release was investigated in freely moving rats. Histology showed that the electrodes were positioned in close proximity to the CA1 pyramidal cells. Rats that were subjected to electrical stimulation did not only behave differently to their sham stimulated counterparts, but also had significantly higher plasma corticosterone levels. Prior treatment of rats with DHPA significantly reduced hippocampal noradrenaline content, but had no effect on electrically stimulated corticosterone release. These data suggested that excitation of CA1 pyramidal cells may lead to increased corticosterone release and that a significant decrease in hippocampal noradrenaline concentration was not able to alter this corticosterone response.

ACUTE AND CHRONIC INTERMITTENT FOOTSTOCK DIFFERENTIALLY INDUCES FOS EXPRESSION IN THE PARAVENTRICULAR NUCLEUS AND ITS EXTRAPARAVENTRICULAR AFFERENTS. P.J. BHAGWANDIAL, P.A. LINT, C.A. Arias and P.E. Swedencot. The Salk Institute, La Jolla, CA 92037

Immuno-localization of Fos protein was coupled with immuno- and hybridization histochemistry and/or axonal tracing techniques in order to dissect acute and chronic effects of intermittent footstock on stress-related neuroendocrine neurons. Exposure to a single 10 min footstock session induced maximal Fos-ir in the paraventricular nucleus of the hypothalamus (PVH) at 2 h after the challenge; at this time point, Fos induction in the paraventricular division of the PVH was localized principally to cells expressing the c-fos immediate-early factor mRNA, while that in the magnocellular neurosecretory system was detected predominantly in corticotrophs. Relative to controls, treated rats, adrenorelated neurons replaced with low levels of corticosterone showed enhanced Fos induction in response to acute footstock in all PVH compartments. Extrahypothalamic cell groups displaying prominent Fos induction in response to acute footstock included catecholaminergic neurons in the nucleus of the tractus solitarius and ventromedial nuclei, the lateral parabrachial nucleus, the ventral lateral periaqueductal gray, and circumventricular structures of the anterior and median tuberal nucleus, septal nucleus and infralimbic cortical areas. Rats bearing retrograde tracer deposits in the PVH and sacrificed 2 or after a single footstock session displayed Fos induction in retrogradely labeled neurons found principally in mediad catecholaminergic cell groups, and secondarily in the lateral septal and medial amygdaloid nucleus. Rats subjected to chronic intermittent stress (2 X 50 min/day for 7 days) and sacrificed 2 h after the final footstock session, displayed patterns of Fos expression that were similar to those seen acutely. Fos expression in chronically stressed animals sacrificed on the day following the final footstock session was not distinguishable from non-shocked control levels. These results suggest that the patterns of cellular activation seen in rats exposed acutely and chronically to a footstock challenge are similar. Mediodl catecholaminergic cell groups are most strongly implicated as candidate afferent mediators of this stressor's effects on endocrine hypothalamic mechanisms.
HYPOTHALAMIC-PITUITARY-ADRENAL AXIS REGULATION II

386.3
HYPOTHALAMIC GALANIN-LIKE IMMUNOREACTIVITY AND ITS GENE EXPRESSION IN RELATION TO CIRCULATING CORTICOSTERONE. A. Abakahyu*; H. J. Chae and S. F. Leibowitz. The Rockefeller University, New York, N.Y. 10021.

The hypothalamic galanin peptide (NPY) and galanin (GAL), known to stimulate feeding behavior, have differential effects on the release of the adrenal steroid, corticosterone (CORT), with NPY enhancing and GAL inhibiting its secretion. In terms of the feedback effect of CORT on endogenous peptide, previous studies have shown that GAL has the potential to produce a reduction of neuropeptide Y (NPY) in specific hypothalamic areas, namely, the paraventricular (PVN) and arcuate (ARC) nuclei, as well as the locus coeruleus (LC). This investigation examined changes in GAL gene expression and peptide levels in relation to circulating CORT in male, Sprague-Dawley rats. Using radioimmunoassay (n=30, 10/group) and in situ hybridization (n=18, 6/group) techniques, this peptide was examined in 3 groups of rats that received either sham surgery, adrenalectomy (ADX), or ADX + CORT replacement. The results showed a clear, site-specific change in GAL in relation to circulating CORT. A loss of CORT after ADX caused a dramatic decline in GAL peptide (<0.05% < p < 0.05) and GAL mRNA levels (<35% < p < 0.05) in the ARC and also peptide levels in the median eminence (<40%, < p < 0.05). Other hypothalamic areas, including the PVN, showed no change. In the brainstem, a similar effect after ADX was seen in the dorsal raphe nucleus, but not in the LC. The data from these brain areas of ADX rats were restored by s.c. CORT implants, which had no impact on GAL in other brain sites. These findings show GAL-synthesizing neurons to respond differently from NPY neurons in relation to circulating CORT.

386.5
Serotonergic denervation attenuates hippocampal mineralocorticoid (MR) and glucocorticoid (GR) receptor gene expression, whereas 5-HT decreases neuronal GR at least in primary culture. The 5-HT1 receptor subtype(s) involved in these effects are unknown. We examined the effects of the 5-HT1A receptor agonist, 8-OHPAD, upon hippocampal MR and GR gene expression in rats bearing central 5-DHT lesions and controls. Rats, pretreated with desipramine, were given 5,7-DHT injected into the dorsal hippocampus (1 mg/kg, 2/4 days later before the electrophysiological examination) or saline for 2 days. Controls (vehicle icv) were given saline or 8-OHPAD. 5-DHT lesions decreased hippocampal MR (~20% fall in CA1 and ~10% fall in the dentate gyrus) and CA1 and CA2 mRNA expression. In 5,7-DHT-lesioned rats, 8-OHPAD administration reversed the fall in hippocampal MR mRNA expression, most notably in CA1. 8-OHPAD had no effect on MR gene expression in controls. By contrast, 8-OHPAD enhances in hippocampal GR mRNA expression in 5,7-DHT-lesioned rats (dentine gyrus, 42% fall; CA1-2, 52%). Moreover, in controls, 8-OHPAD also decreased GR mRNA expression in CA1 (31% fall) and CA2 (39%). The attenuation of hippocampal MR gene expression by 5-HT denervation can be partly reversed by 5-HT1A receptor agonists, whereas the attenuation of GR gene expression does not appear to be due to loss of 5HT1A receptor activation. Whether the 8-OHPAD-related decrease in GR mRNA expression is directly mediated or reflects indirect actions, such as increased corticosterone secretion, remains to be determined.

386.6
Serotonin and corticosteroids interact in the hippocampus, affecting electrophysiological, neuroendocrine and behavioural parameters. Chronic m-catenylhexamethylenamine (MDMA) administration causes selective neurodegeneration. This decrease is most pronounced in the paraventricular nuclei (PVN) and extends to the hippocampus and subiculum. In the hippocampus, 5-HT receptors modulate neurotransmitter release and are implicated in various types of neurodegeneration. Similarly, 5-HT receptors are implicated in the development and progression of Alzheimer's disease. Following MDMA-induced 5-HT receptor gene expression changes in the hippocampus, we examined the effects of acute MDMA on hippocampal MR, GR, and 5-HT3 receptor mRNA expression using in situ hybridization. Rats were killed 6 h after MDMA (20 mg/kg, i.p.). Hippocampal neuronal GR mRNA expression was decreased in the dentate gyrus (DG) (15% fall) and CA1 (20%) and CA3 (24%); all p < 0.05. In contrast, MR mRNA expression was increased in the DG (69% rise), CA1 (41%), CA3 (55%) and CA4 (52%); all p < 0.01. 5-HT3 receptor mRNA expression was decreased in CA3 of the dorsal (20%, p < 0.01) and ventral (30%, p < 0.05) hippocampus. Increased MR and decreased 5-HT3 receptor gene expression in the hippocampus probably reflect acute MDMA-induced 5-HT release. Down-regulation of GR gene expression might be a consequence of these changes. These results support our previous findings suggesting that 5-HT controls hippocampal corticosteroid receptor and 5HT3 receptor gene expression.

386.7
ALTERED EXPRESSION OF GLUT1 & GLUT3 GLUCOSE TRANSPORTERS IN NEUROHYPOPHYSIAL EINHEERODYSTASIA. Kang Li, Susan J. Vannucci, Ellen Koelbel, Fran Maher and Jan A. Simpson* Bethesda, MD 20892 and Hershey, PA 17073.

The neurohypophysis (NH) is an extension of the central nervous system which contains axon terminals of vasopressin- and oxytocin-secreting neurons of the hypothalamus and glial-like pituicytes, but lacks a blood glucose (GLUT1 and 45 kDa GLUT3) detected in the NH. Progressive dehydration due to water deprivation increases vasopressin (VP) secretion in rats; dehydration increases NH glucose utilization. This study examined the effects of progressive dehydration and subsequent rehydration on NH GLUT1 and GLUT3. Dehydration was achieved in adult (250g) male rats by water deprivation for 1, 2 and 3 d. All dehydration other rats had their water returned and were studied at 1, 2 and 3 days following rehydration. NH GLUT1 & 3 concentrations were determined in these rats using a [125I]-labeled 6D1 antibody with specific anti-terminal antibodies. With progressive dehydration, hemocrit increased and plasma VP levels, measured by RIA, increased 4-fold by 3 days in dehydration in NH VP. The concentration of GLUT1 was increased by 18% and 44% by 2 and 3 days of dehydration and GLUT3 was increased by 42% & 55%. With water repletion, physiological and biochemical differences to the control group were restored within 2 days. However, the levels of both GLUT1 and GLUT3 remained elevated, even at 3 days of rehydration. The results of this study suggest that NH glucose transporters are rapidly upregulated in response to the metabolic stress of dehydration but their turnover is apparently slower.

386.8
MODULATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS IN BIPODS. L. Michael Proctor* and C. William Field, Dept. Zoology, Univ. of Washington, Seattle, WA 98105

Recent evidence suggests that some avian species can modulate corticosterone (B) release in response to a standard stressor paradigm (capture and serial blood sampling). Stress-induced release of B varies throughout the year and appears dependent upon the animal's physiological state. In one species (white-crowned sparrow, Zonotrichia leucophrys), B output is inhibited during molt. We tested whether this inhibition results from adrenal or peripheral mechanisms by injecting various stimulating factors into molting white-crowned sparrows and comparing B release to simultaneous release from animals (Genus luteola), a species not known to modulate their glucocorticoid response. White-crowned sparrows from eastern USA were housed in outdoor aviaries until the onset of molt. Blood samples were taken at 15-min intervals. Blood was injected, bled, and released. Intravenous ACTH stimulated B release in the sparrows, indicating that the adrenal cortex can still respond to an ACTH signal during molt. CRF, on the other hand, failed to stimulate B release in the sparrows, but enhanced B release 300% in the pigeon compared to vehicle-injected controls. Similar suppression of vasopressin and mesencephalin (AVT and MT - the avian equivalents of vasopressin and oxytocin, respectively) also failed to stimulate B release in sparrows whereas AVT caused a 200% increase in the pigeon. This suggests that CRF and AVT enhance B secretion than to either hormone alone. These data suggest that the inhibition of B release seen in white-crowned sparrows during molt may be mediated by pituitary insensitivity to an ACTH antagonistic signal but not to adrenal insensitivity to ACTH.
386.9

PERIPHERAL NERF ADMINISTRATION INCREASES HYPOTHALAMIC C-FOS EXPRESSION. F. Passarelli, R. Nicolai*, F.R. Butterelli, D. Bablioni, S. Scaccioni*, G. Cipriani*, and I. Angelucci*. Dept. of Neurosci., Univ. of Rome “La Sapienza” and #Farmacologia, 2°, Univ. of Rome “La Sapienza”

We have recently demonstrated in the rat the hypothalamic involvement in the adrenocortical activation induced by NFG (Scaccioni S., 1993). The c-fos gene, expressed at low levels in most cells types, is induced rapidly in response to a variety of extracellular stimuli in the periphery and in the CNS. We have also observed whether NGF administration was followed by an induction of hypothalamic c-fos mRNA expression. Freely moving male Wistar rats were injected through a permanent jugal cannula with either 1 or 5 mg/kg of mouse ENGF (kindly donated by Dr. J.R. Perez). Pol, Univ. of Texas, Galveston, Texas) or vehicle. The adrenal glands and the hypothalamus were isolated 20 min after injection and total RNA was extracted by the guanidinium thiocyanate–cesium chloride method. The levels of c-fos and beta-actin mRNAs were measured in each sample using a RT-PCR. Compared to vehicle in the hypothalamus a 2-fold c-fos mRNA increment was observed after 5 mg/kg of NGF administration but not after 1 mg/kg. The adrenal gland mRNA c-fos enhancement was detected after 1 and 5 mg/kg NGF injection. These results confirm that the hypothalamic is involved in the adrenocortical activity induced by NGF and that NGF is a target of messenger pathway. (Supported by CNR grant 93.0447.PF40 to Scaccioni S.)

386.10


In fetal sheep, implants of corticosteroids adjacent to the paraventricular nucleus of the hypothalamus suppress AVP and CRH expression. These effects of glucocorticoids are mediated by actions of glucocorticoid receptors within the paraventricular (PVN) neurons. Under physiological conditions, corticosteroids could influence the function of the hypothalamus via actions to the PVM afferent neurons as well. To assess this possibility, we determined whether the catecholaminergic afferents to the fetal medial paraventricular (A1/C1) and, n. solitari tract (A2/C2) possessed immunoreactive type II glucocorticoid receptors. To accomplish this, fluorogold (FG) was stereotaxically injected into the PVN of 5 fetal sheep (120A). After 3 weeks, each fetus was removed by Caesarian section, administered an overdose of anesthesia and perfused intracardially with fixative (using anti-BUSG), tyrosine hydroxylase and FAU was performed in the brainstem. Staining of the hypothalamus for FAU, GRII, and CRH verified the accuracy of tracer injection sites and the potential sites of innervation within the CRH neurons of the PVN. The dominant catecholaminergic innervation of the PVN arises from the A1/C1 and A2/C2 cell groups. In the A1/C1 cell group, 66% of the catecholamine neurons that project to the PVN in the A2/C2 cell group 73% of the neurons were GRII, Triple labeling of tyrosine hydroxylase, GRII and FAU confirmed that approximately half of the GRII catecholamine neurons in both regions projected to the PVN. These data suggest that glucocorticoids may act on both the CRH neurons of the hypothalamus as well as on catecholamine afferents to the PVN. (Supported by NIH HD 21350)

386.11

GLUCOCORTICOID MODULATION OF C-TYPE NERFATICUR PEPTIDE mRNA LEVELS IN PREFRONTAL AREA AND LIMBIC STRUCTURES. M. C. Langub, Jr.*, D. Bucker and J.P. Herman, Dept. of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, KY 40536-0084. Lesion and anatomical analyses suggest that the ventral subiculum (VSUB) plays a major role in hippocampal inhibition of the hypothalamic-pituitary-adrenocortical (HPA) axis. The present study was designed to examine the role of VSUB in stress-induced hypothalamic, neurotransmitter and neuroimmune stress responses. Groups of rats received bilateral ibotenic acid lesions of the VSUB (n=19) or injections of saline vehicle (n=17). All animals underwent analysis of behavior related behaviors in an open field, and were subsequently divided into three groups for analysis of endocrine and immune responsivity to acute restraint (60 and 120 min). IBO rats exhibited enhanced hypothalamic responsivity to open field exposure, manifest as increases in freezing behavior and decreased ambulation. IBO lesion increased stress-induced ACH secretion (n=10) but not 120 min stress induction; no changes were seen in baseline release. Restrain stress caused marked decreases in splenic lymphocyte proliferation following 60min of restraint, as measured by nile nitrogen assay; however, no differences were found between groups. The results suggest that VSUB damage affects both emotional responsivity and HPA activation following exposure to a stressful situation, yet do not impact either basal ACTH release or immune reactivity in a long-term fashion. The VSUB lesion appears to bias behavioral predispositions in a direction suggesting enhanced stress responsivity. Effects of VSUB lesion on HPA activation may therefore be mediated by alterations in salience of stressful stimuli, rather than modulation of glucocorticoid negative feedback. Supported by MH46908.

386.12

BEHAVIORAL, NEUROENDOCRINE AND NEUROIMMUNE ANALYSIS OF HIPPOCAMAL STRESS INTEGRATION. J.P. Herman*, C.M. Dolgas and S.L. Carlson, Dept. of Anatomy and Neurobiology, Univ. Kentucky Medical Center, Lexington, KY 40303-0984.


Activation of the hypothalamic-pituitary-adrenal (HPA) axis causes an increase in the circulating levels of glucocorticoids (eg, during stress). Glucocorticoids feed back onto the brain to inhibit the secretion of corticotropin releasing factor (CRF) and suppress HPA activation. To determine whether glucocorticoids feed back directly onto hypothalamic neurons of the hypothalamus, we studied the effects of the glucocorticoids, dexamethasone, on the membrane electrical properties of identified neurons in the rat hypothalamic paraventricular nucleus. The effects of dexamethasone on specific PVN cell populations were studied using intracellular recordings, dye injection and immunohistochemistry in the hypothalamic slice preparation. Bath application (30 min) of dexamethasone (10^-6 M) caused a >44-AE increase in voltage hyperpolarization with an increase in input resistance in low-threshold spiking (LTS) cells, or putative paravocellular neurons, and a small depolarization (2-mV) with a decrease in input resistance in non-LTS cells, or putative magnocellular neurons. The identification of these cells was verified anatomically with biocytin injection and subsequent immunohistochemical processing using antisera to CRF, oxytocin, vasopressin or neuropeptide. These results suggest that glucocorticoids have a direct inhibitory effect on parvocellular neurons and a weak excitatory effect on magnocellular neurons of the hypothalamic PVN.
EVIDENCE FOR GLUCOCORTICOID REGULATION OF VASOPRESSIN V1a RECEPTOR GENE EXPRESSION IN HYPOTHALAMIC-PITUITARY-ADRENAL AXIS REGULATION II

386.15

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The brain is intimately associated with the hypothalamic-pituitary-adrenal axis. We have isolated a genomic clone from a rat testis library encoding a putative vasopressin V1a receptor. Sequencing of the clone revealed the presence of at least five potential introns. A possible tissue-specific expression of this gene has been detected in rats with PHX-induced diabetes, in which vasopressin V1a receptors are upregulated in the hypothalamus. Support provided by the VA and Pharmacological Sciences Training Grant 587048.

386.16

ADRENOCORTICAL EFFECTS ON TYPE I CALMODULIN-DEPENDENT ADENYLYL CYCLASE mRNA LEVELS IN RAT BRAIN.

M. G. Mengo* and B. S. McF岐, Lab. Neuroendocrinol., Rockefeller Univ., NY, NY 10021

The hypothalamic-pituitary-adrenal axis has pronounced effects on calmodulin-dependent (CaM) adenylyl cyclase (AC) enzyme activity, measured in rat brain tissues. In particular, hippocampal AC activity shows dexamethasone- and adrenocortisol-stimulated release, an effect abolished by adrenalectomy. Type I ACmRNA is present in both hypothalamic-pituitary regions and in brain. This study investigated whether adrenocortical effects on AC activity were paralleled by changes in the expression of AC mRNA. The results of this study support the hypothesis that the hypothalamic-pituitary-adrenal axis regulates AC activity in rat brain. Supported by grant 5NH1256.

386.17

MINERALOCORTICOID RECEPTOR mRNA VARIANTS IN THE DEVELOPING HIPPOCAMPUS: DISTRIBUTION AND CORTICOSTEROID REGULATION


Our laboratory has previously reported an MR DNA clone from rat hippocampus (HC) which shares a high degree of homology with the kidney MR DNA clone. These two clones differ significantly at the 5' untranslated region (5'UT) and have been named alpha (a) (kidney clone) and beta (b) (HC clone). Most recently, a third HC 5'UT DNA clone was isolated which we termed gamma (γ) (Endo 133-234). Studies have shown that these 5'UT regions are located on separate exons of a single gene and give rise to three mRNAs that encode the same protein. We are interested in investigating the translation of these distinct mRNA forms and their glucocorticoid regulation in the developing rat HC. Last year we presented the α and β HC mRNA distribution in adult rats and we have expanded the in situ hybridization study to younger animals and included the γ HC variant in our analysis. In general, we find that the α and β expression increases significantly in all HC regions during the first 2 weeks of life. Whereas α levels remain constant thereafter, β expression decreases markedly in the piriform cortex. In contrast, γ is highly expressed in all regions early during development. In the dentate gyrus this high level of expression is retained until adulthood when lower levels are detected. Adrenalectomy results in up-regulation of the α 5'UT form in all ages. Our results, show that the α and β 5'UT forms achieve an adult pattern of distribution in the HC after the Stress Hypo-Responsive Period. The γ 5'UT form may be important for proliferation and differentiation of hippocampal neurons. Supported by NIMH MH40976 and MH14225.

387.1

GABA TURNOVER IN MICRODISSECTED BRAIN REGIONS DURING THE RAT ESTROUS CYCLE.

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GABAergic neurons terminating in the rostral and medial basal hypothalamus are positively regulated by testosterone, an action that may play an important role in the negative-feedback action of testosterone on hypothalamic GnRH release in the male rat. The present study determined whether endogenous changes in gonadal steroids during the estrous cycle are associated with changes in GABAergic neuronal activity measured in tissue punches from steroid-sensitive brain regions. Animals were decapitated at 0800 and 1700 h on diesterrous day 1, and 0900, 1300 and 1700 h on proestrus, either without treatment, or 60 mm without inhibition of the GABA degrading enzyme GABA-transaminase by amino- oxyacetic acid (AOAA, 100 mg/kg). The rate of accumulation of GABA in the tissue after AOAA was taken as an index of GABA turnover. In brain regions examined so far, there was no change in GABA turnover between the morning and afternoon on proestrus, or between the morning of diestrus and the morning of proestrus. In the diagonal band of Broca at the level of the OVLT, GABA turnover was significantly reduced in the afternoon of proestrus compared with morning. By contrast, in the central portion of the MPN, no changes were apparent at any of the timepoints examined. Analysis is continuing in the median eminence, ventromedial nucleus, septal nuclei, and hippocampus. Supported by NHLBI, NIMH, and NIDA. 5NH12369.

387.2

EFFECT OF CHRONIC MORPHINE TREATMENT ON NORDADERENERGY MODULATION OF GT1-7 NEURONS. M. A. Lavazza*, T. S. King, J. G. Hensler, and X. Chang. Departments of Pharmacology, Psychiatry, and CRBII, The University of Texas HSC, San Antonio, TX 78284

Stimulation of noradrenergic, serotonergic, and opiate receptors are known to regulate release of hypothalamic GnRH, but it is not known whether the receptors exist on GnRH neurons or on other cell types that modulate GnRH release. GT1-7 neurons, which synthesize and secrete GnRH, were immortalized in culture from transgenic mice. The purpose of this study was to examine the possible interaction between GnRH neurons and GABAergic neurons that modulate GnRH release. Supported by the NIH and the Texas HHSC. 5NH12369.
387.3 GONADOTROPIN-RELEASING HORMONE (GnRH) RELEASE IN MAMMALS: THE ROLE OF THE HYPOTHALAMUS

387.4 FOOD DEPRIVATION ENHANCES ESTRADIOL-INDUCED LH SURGE IN THE SCID MOUSE

387.5 SUSPENSION OF LUTEINIZING HORMONE SECRETION IN FOOD-RESTRICTED LACTATING RATS: EFFECTS OF OVARIECTOMY AND ADIPOCYTOKINE TREATMENT

387.6 BLOCKADE OF PREOVULATORY LH SURGES WITH A NEUROPEPTIDE Y RECEPTOR ANTAGONIST, PP43:

387.7 ACUTE INHIBITION OF LUTEINIZING HORMONE SECRETION IN RESPONSE TO ETHYL-β-CARBOLINE-3-CARBOXYLATE (β-CEE) AND DIAZEPAM TREATMENTS IN CASTRATED MALE RATS

387.8 SODIUM NITROPRUSSIDE-INDUCED RELEASE OF NITROGEN MONOXIDE (NO) FROM PROCEPHALON MEDIAN Eminence (MEd) RECEPTORS: DIRECT EFFECTS ON HIPPOCAMPAL NEURONAL FUNCTION

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
PARTIAL SURGES OF LUTEINIZING HORMONE (LH): A NEUROENDOCRINE MECHANISM FOR PROLONGED EROTIC CYCLES IN FEMALE RATS AND HUMANS

The LH surge that stimulates ovulation is triggered by a daily neural signal that has been adequately provoked by ovarian steroids. This provocative signal is characterized by (1) a rise above diastolic LH level and (2) an accelerated rate of LH secretion. 7 ± 2 hypophysial pulsatility sites in two chow-fed rats collected seven times a day, hourly, from each female on four proestrus days. Steroid levels were assessed in daily samples collected at lights out throughout two estrous cycles. In 57 full LH surges, the rate always preceded the acceleration in the rate of LH secretion. Nonetheless, the time of the rise did not predict the latency to the acceleration. These two independent characteristics of the LH surge correlated with different steroid levels. Moreover, in addition to eight surges LH rose above diastolic LH levels but never achieved the acceleration typical of the rising limb of the LH surge. We termed these partial LH surges and hypothesized that they could be a result of the ovarian follicle without ovulation, thus initiating prolonged estrous cycles characteristic of estrous cycle distributions in non-mammalian species. The females whose full LH surges had a delayed acceleration were the ones that might have possible partial LH surges.

In addition, the steroid correlates of partial LH surges were the same as those that were present.

Supported by MH10127 (SG) and MERIT award R37 MH17488 (MM).

DOFAMINE AND THE INHIBITION OF LUTEINIZING HORMONE PULSES IN RATS. THE SIGNIFICANCE OF HYPOTHALAME. P. P. O. Hofsten and C. W. Cost. Division of Biological Sciences, University of London, W1, U.K.

Previous work has indicated that a reduction in body temperature can be the primary factor in the suppression of diurnal cycles of hypothalamic function. Since it has been demonstrated in independent studies that centrally administered dopamine can induce hypothermia and inhibit LH pulses, we have examined the effects of the dopamine receptor agonist and antagonist on these parameters. In some of the studies the agonist was administered after a dopaminergic antagonist and at others it was given at an interval before the LH pulse. The results showed that dopamine antagonists could decrease the LH pulse size and that dopamine agonists could increase it. The antagonist and agonist were given by push-pull cannulae and the results showed that the dopamine antagonist decreased and the dopamine agonist increased the LH pulse size.

The results of these experiments suggest that dopamine may be an important factor in the control of LH release in the rat.

PRESENCE OF FUNCTIONAL OPIOID BINDING SITES ON THE GT-1 LH SECRETING CELLS.

B. Maggi, F. Fimpanelli, M. F. Pia, Dept. of Endocrinology, University of Milan, Italy.

The presence of functional opioid binding sites on the GT-1 LH secreting cells was studied with the use of [3H]rogue, [3H]DADLE, and [3H]DPDPE. The results showed that the GT-1 LH secreting cells were sensitive to the effects of these opioids. The presence of opioid binding sites on the GT-1 LH secreting cells suggests that opioids may play a role in the regulation of LH release.
Castration decreases mRNA levels encoding glutamic acid decarboxylase (GAD), the rate-limiting enzyme for aminobutyric acid (GABA) biosynthesis, within regions of the brain containing GABAergic cells. Closer examination has revealed that the majority of GAD-labeled cells in the MPOA are coexpressive with the region previously described by Gorski and colleagues as the SDN-MPOA. The objective of the present study was to examine the effects of castration on expression of GAD mRNA specifically within this highly stereotyped-sensitive area of the brain. Brain sections from 2-6x6 mm blocks were taken from anesthetized rats, and 48 hours postcastration examined by confocal microscopy to evaluate GAD mRNA content, in an attempt to determine if GAD is upregulated in the absence of testosterone. We observed that the expression of GAD mRNA increased significantly in the PVN of castrated rats compared to controls. In the MPOA, no significant change was observed in GAD mRNA expression. We have concluded that the increased expression of GAD mRNA in the PVN is a result of castration, and that this may be due to the loss of inhibitory effects of testosterone on GAD mRNA expression. These findings have implications for understanding the mechanisms underlying the regulation of GABAergic neurotransmission in the brain. Further studies are required to determine the precise role of GAD in regulating GABAergic activity in these regions, and to identify potential therapeutic targets for the treatment of disorders involving GABAergic dysfunction.
388.5 SYNAPTIC BURST ACTIVITY IN HYPOTHALAMIC NEURAL NETWORKS. W. Muller* and D. Swadulla MPI biophysics. Chemie, D-37077 Goettingen and Pharmakol. Institut, Universitaetsstr. 22, D-39106 Erlangen, FRG.

Burst activity is important for the release of hormones from central neurons. In dissitributed (picrotoxin 20 μM) networks of hypothalamic neurons in culture, neurons exhibit 'rhythmic' burst activity that is blocked by the glutamate receptor antagonist CNQX (10 μM; D-cyclohexyl-2-amino-2-phenylglycine 2.3-μM). Using Ca2+ imaging on Fura-2/AM loaded cultures and patch clamp techniques we found synchronous increases in [Ca2+]i in a large majority of neurons (>85%) that corresponded to bursting activity recorded from a single neuron. In 20 μM CNQX, none of the neurons showed oscillations in [Ca2+]i.


In mammals corticotropin-releasing factor (CRF) regulates the pituitary release of adrenocorticotropic hormone (ACTH). In teleosts CRF is also thought to stimulate the release of ACTH, but in addition, seems to be involved in the regulation of immune activity. We localized CRF-like immunoreactivity in the brain and pituitary of hatchery-reared juvenile, 12 to 15 months, chinook salmon (Onchorhynchus tshawytscha). Fish were perfused with Bouin's fixative (10%) Submammary, brains from fore and sections at 30 microns were then incubated with polyclonal CRF antiserum for 48 h. Incubation for less than 48 h resulted in little or no staining. Precipitation of the antibody with CRF eliminated all observed staining.

388.7 cFOS AND cJUN ARE EXPRESSED IN CULTURED HYPOTHALAMIC TRH NEURONAL SYNTHESIZING NETWORKS BY GLUCOCORTICOIDS. L.G. Low and J.M.D. Jackman.* Division of Endocrinology Brown University, Rhode Island Hospital, Providence, RI 02903.

There is evidence that glucocorticoids (GC) regulate the hypothalamic-pituitary-thyroid axis. Although GC have been reported to reduce proTRH mRNA levels in the thyrotrpic area of the hypothalamus in vivo, it has not previously been feasible to determine the direct effect on hypothalamic TRH neurons in vitro. Accordingly, we have studied the effect of GC on proTRH mRNA levels in hypothalamic cultures (Jackman MD et al., 1994) and the possible involvement of the protooncogenes c-fos and c-jun in this process. A non-isotopic double label in situ hybridization histochemistry method was used to examine the possibility of colocalization of TRH with c-fos and c-jun mRNAs. We demonstrated that the mRNA for TRH is coexpressed with both c-jun and c-fos in hypothalamic cells. Treatment with dexamethasone (Dex) enhanced the cell signal for both TRH and c-fos/c-jun, and also increased the number of cells coexpressing their mRNAs. Following the use of Image Analysis, Dex 10-8M enhanced mRNA of TRH 133% (p<0.01; n=6), c-jun 131% (p<0.01; n=6) and c-fos 148% (p<0.01; n=6). We also applied 32-P-labeled RNA Blotting and Image Analysis to measure the steady state levels of c-fos and TRH mRNA. The data revealed an enhanced expression of c-fos 3.1 fold, c-jun 4.2 fold and proTRH 3 fold (n = 4, p<0.01). Nuclear run-on analysis of transcription was performed to measure the effect of Dex. The results showed that Dex 10-8M increased the transcription activity of c-fos 3.4 fold, c-jun 5 fold and proTRH 7.7 fold (n = 3, P<0.01). Conclusion: 1. Dex enhances the expression of proTRH mRNA as well as c-fos/c-jun mRNAs in hypothalamic neurons in vitro. 2. Dexamethasone increases the transcription activity of c-fos, c-jun and proTRH in hypothalamic in vitro. 3. The existence of c-fos/c-jun in TRH neurons and the increase of their transcription activity by Dex suggests that these protooncogenes may mediate the effect of glucocorticoids on TRH gene transcription.

The macrophage, a critical component of the immune response, is profoundly affected by stress. We have previously reported stress-induced interleukin 1 (IL-1), prostaglandin (PGE2), and Ig antigen alterations (Jiang, et al., 1990). We now report findings for increased interleukin 6 (IL-6) and tumor necrosis factor (TNFα) following exposure to stress (p<0.05, p<0.01, respectively). In addition, preliminary data show increased specific binding (Bmax) and/or increased binding affinity (Kd) for substance P (SP) to the peritoneal macrophages from stressed mice. The stress paradigm for all work involved subjecting male C57Bl/J6 mice to 5-minute swim tests in 10°C (+/−2°C) water twice daily for 4 days. Cytokine concentrations were determined by ELISA, and SP binding was assessed with fluorescein- and 125I-labeled SP. Specificity and affinity were determined by competitive displacement with unlabeled SP. Results from this work, together with recent findings for stress-induced increases in immunoreactive SP in the peritoneal wash fluid, suggest that perhaps SP interacts with cell-surface receptors to mediate the various functional alterations in macrophages following stress.


Exposure to inescapable tail shock (IS) results in decreases in anti-KLH Ig. The mechanisms responsible for this change are not well understood. The following studies assess the possible involvement of a putative endogenous antinociceptive benzodiazepine ligand in modulating IS-induced suppression of anti-KLH Ig.

Naturally occurring compounds (e.g. diazepam binding inhibitor, DBI) have been shown to increase immunosuppression and bind to both the central-type (CBR) and, the peripheral-type (PBR) benzodiazepine binding sites, & modulate the immune system. We examined the effect of CBR & PBR blockade during IS on the IS-induced reductions in anti-KLH Ig. Rats (13g) were immunized with KLH (200µg) i.p., injected with either RO15-1788 (RO 10 mg/kg; CBR antagonist) or PK1195 (PK 8 µg/kg; PBR antagonist) & exposed to 100 ± 6.6 mV of IS or returned to their home cages (HC). anti-KLH IgG levels were measured using ELISA. RO did not block the IS-induced decrease in anti-KLH IgGs, whereas PK did. Since prior work has suggested that glucocorticoids may play a role, we blocked IS-induced suppression with a CBR antagonist (RO15-1788). We found that IS-exposed rats had lower levels of anti-KLH Ig, compared to IS-exposed + RO15-1788. These results suggest that the IS-induced decrease in anti-KLH Ig may be mediated by the CBR system.


Stressors can result in changes in immune function. Exposure to inescapable tail shock (IS) results in long-term decreases in anti-KLH IgGs. The mechanisms responsible for this change are not understood, but evidence suggests an involvement of altered T cell subpopulations. The following studies examined these earlier observations. Rats were immunized with KLH (200µg IP) and exposed to IS (n = 6) or returned in their home cages (n = 6). Lymphocytes from the mesenteric lymph node (MLN), cerebral lymph node (CN) and spleen (SPL) were obtained 48, 72, 96, and 168 hrs after IS termination. The percent of CCR+. TCD4+, CCR+. TCD8+, CD45RC-. CD4+ (Th1-like) and CD45RC+. CD4+ (Th2-like) lymphocytes were assessed at each time point for each tissue using two color flow cytometry. The number of KLH specific splenic B cells was also measured 7 days (168 hrs) after KLH+IS using ELISPOT. IS resulted in a complex pattern of changes. The greatest shifts occurred immediately after IS + KLH and 96 hrs (4 days) after IS + KLH. IS resulted in an increase in CD4+ T cells and Th1-like cells per gram of wet tissue only in the spleen. Four days after IS + KLH, there was a decrease in Th1-like MS cells and an increase in Th2-like MS cells. Seven days after IS + KLH there was a reduction in the number of KLH specific splenic B cells. These changes in T cell phenotypes could be involved in causing the IS-produced reduction in the formation of KLH specific B cells and alterations in immunoglobulin levels and isotypes (IgG1 & IgG2). NIH-MH54954


Inescapable tail shock (IS) suppresses IgG response to KLH. Evident suggests that alterations in early processing events such as antigen transport & processing by macrophages may be involved. Someors can alter Ms function & can make Ms suppressive. The following studies examined the role of Ms in stress-induced decreases in anti-KLH IgGs. First, stress-induced changes in Ms numbers were investigated immediately after IS + KLH in the peritonal cavity, the mesenteric lymph nodes (MS), cervical lymph nodes (CN), & the spleen (SPL). Cells were labeled with anti-ED1 (Ms specific antibodies) & anti-CD11c (a anti-CD11c) followed by a second color flow cytometry. IS decreased ED1+ peritoneal cell number & ED1+ splenic cell number immediately after IS + KLH. In contrast, the number of ED1+ cells in both MS & CN increased after IS + KLH. If the number of IS+ED1+ cells reflects an increase in a suppressive Ms population, then inhibition of these cells should result in a potential increase in immune system function. Anti-CD11c (a) (12g) were injected intraperitoneally (IP) with either gandoline chloride (GdC3; 7.5 mg/kg, a phagocyte inhibitor), or saline. Twenty-four or 72 hrs after GdC3, rats were immunized with KLH (200µg) either IP, which includes the draining nodes (MS), or intravenously (IV), which bypasses the draining nodes. The Ig response to KLH, measured using ELISA, was found to be enhanced in IP immunized rats both 24 hrs & 72 hrs after GdC3. In contrast, GdC3 had no effect in IV immunized rats 24 hrs after GdC3, & slightly suppressed the Ig response in these rats 72 hrs after GdC3. It appears, therefore, that Ms can have a suppressive effect on Ig production in response to IP KLH & that IS-induced decreases in anti-KLH Ig production may be due to increased suppressive Ms in the MS. The importance of IS-induced decreases in peritoneal and SPL Ms is currently being investigated. NIH-MH540545.

389.8 CONDITIONED STRESS-INDUCED ALTERATIONS IN IMMUNE FUNCTION IN LACTATING RATS. N. Shapiro*, M.A. Porzene, G.D. Hoffman, B.S. Rabin. Deps. of Clinical Immunopathology & Neurobiology, University of Pittsburgh Med. Center, Pittsburgh, PA Stress-induced CNS and leukocyte alterations are increased in lactating females. We determined whether lactating rats were similarly resistant to stress-induced alterations in immune function. Sprague Dawley female rats were exposed to either 2 sessions of 16 footshocks (1.6 mA, 5 sec) where shock was paired with a tone (C+), or were left undisturbed in their homecages (HC). Half of the rats were bred and on day 10 of lactation half the C+ rats were exposed to the same footshocks, but shock was withheld (C−C+), while the other half were left undisturbed (C−HC). Diestrous rats received identical stress treatments as did lactating rats (L). Exposure to these treatments resulted in increased levels of plasma corticosterone (6.8-8.3 µg/dL). Stress levels were elevated in both C+ groups immediately following re-exposure, but C−C+ exhibited lower levels of steroid (34.8 µg/dL) than C−HC (50.1 µg/dL). Spleenocyte xTCR stimulated proliferation was suppressed in C−HC, but remained at nonstress levels in C−C+. Whole blood mitogen responses (PHA, ConA) were suppressed following conditioned stress, but LPS stimulated proliferation was enhanced in both C−HC and C−C+. Mesenteric lymph node (MLN) lymphocyte proliferation stimulated with anti-T cell receptor antibody (aTcR) or ConA was enhanced in C−HC, while this effect was less noticeable in C−C+. These data suggest that lactating rats are resistant to stress-induced suppression of splenocyte proliferation, as these alterations to blood lymphocyte immune function as are NLC controls, and exhibit greater stress-related enhancement of MLN proliferation.

389.11
MONOAMINERGIC, NEUROENDOCRINE AND IMMUNE RESPONSES TO STRESS: THE INFLUENCE OF LATERALIZATION. C. Deluze, B. Delpeigne, J.A. Antelis, L. Bouyer, S. Gessert

Stress responses including modifications of immune reactivity, neuro-endocrine functions, and brain functioning were hypothesized to depend on lateralization. To answer this question, we studied the stress responses in mice selected as left- and right-handers in a paw preference test.

First, restraint stress, known to affect immune reactivity, induced modifications in the mesolimbic dopaminergic (DA) system which are asymmetrically expressed and are related to behavioral lateralization. Indeed, the asymmetry of DA metabolism in the nucleus accumbens observed in left-handers correlated with paw preference scores, disappeared after stress. Conversely, an asymmetry in DA metabolism in the frontal cortex, appeared in stressed animals.

Second, the injection of lipopolysaccharide (LPS) is usually followed by a decrease in T-lymphocyte proliferation, an increase in ACTH and corticosterone plasma levels and an augmentation in brain monoaminergic metabolism and thus may be considered as a stress inducer. The modifications of brain monoaminergic metabolism were asymmetrically expressed and depend upon behavioral lateralization. Plasma corticosterone was enhanced in both left- and right-handers, but plasma ACTH was increased only in right-handed animals. T-lymphocyte proliferation was depressed only in right-handers.

These results show that an immune challenge, which may be considered as a stressor, induces modifications in the activity of the central nervous system, the HPA axis and the immune system, that depend on brain and behavioral lateralization. Therefore, the bidirectional pathways between the brain and the immune system appear to be subject to lateralization.

389.13
ADVERSE EFFECTS OF CHRONIC NICOTINE ON IMMUNE ORGAN ATROPHY IN AN ANIMAL MODEL OF ANOREXIA NERVOSA. A. Chiu, D.M. Anos, H.A. Funk, T.S. Reg* and P.P. Arvidson, Virginia Governor's School for Science & Technology, Hampton, VA 23669; Eastern Virginia Medical School, Norfolk, VA 23501; Veterans Affairs Medical Center, Hampton, VA 23667.

Anorexia nervosa (AN) is an eating disorder associated with excessive activity, weight loss and immunosuppression. Nicotine increases activity, decreases body weight and affects immune function. This investigation determined if nicotine administration affects weight loss and worsens the thymus and spleen atrophy associated with a popular animal model of AN. The model was produced by giving moderately food-deprived rats (1.5-2.5x body food access) free access to running wheels (22.5°/day). Three groups of rats (9-10 rats/group) were injected with either 0, 0.4 or 0.8 mg/kg/day of nicotine (free base; s.c.) for 11 days prior to and throughout the syndrome. Contrary to previous reports, nicotine administration affected weight gain and food intake and rate of weight loss in the model. However, nicotine worsened the thymus atrophy associated with the syndrome. This effect, which was specific to the thymus and did not occur with the spleen, was related to an exacerbation of the relative adrenal hypertrophy that occurs in the syndrome. It is proposed that nicotine stimulates glucocorticoid secretion, which adversely affects the immature T lymphocytes of the thymus but spares the mature lymphocytes of the spleen. These data raise the possibility that, while nicotine may not affect weight loss in AN, it may adversely affect immune function, which is already compromised in the disorder.

389.10

The present study was performed in order to determine whether stress-induced catecholamines release may be correlated with the inhibition of influenza virus-specific (and/or poliovirus-specific) immune responses and specific-induced lymphoproliferation. Unconditioned Thoroughbreds were subjected to sham, psychological or various intensities of exercise stress. Jugular blood was collected at four different time points, before and during each stress test. Triglyceride cultures of 2x10° peripheral blood mononuclear cells were incubated at 39°C with control medium and with various concentrations of norepinephrine and epinephrine. Mitogen. Proliferation was measured by incorporation of tritiated thymidine incorporated into days 0 and 7. Blood samples obtained at the aforementioned 4 time points were also assayed for catecholamines using high performance liquid chromatography. Non-specific lymphoproliferation was significantly decreased during slow or exercise stress. Specific lymphoproliferation declined (P < 0.05) in all but the sham stress test and was increased (P < 0.05) at the end of psychological stress. Circulating concentrations of norepinephrine and epinephrine were increased (P < 0.05) by all stress tests. Thus, stress-induced changes in lymphoproliferation were not related to increased catecholamine concentrations that resulted from psychological and physical stressors.

389.14
IMMUNOSUPPRESSIVE TREATMENT RESTORES EXPLORATORY BEHAVIOR IN AUTOIMMUNE MRL-lpr MICE. Boris Šakić*, Henry Szechtman and Judith A. Denburg. Deps. of Biomedical Sciences and Medicine, McMaster Univ., Hamilton, Canada L8N 3S5.

Various behavioral deficits of unknown etiology accompany systemic autoimmune diseases in humans. Autoimmune MRL-lpr mice show a variety of behavioral deficits in comparison to undiseased age-matched controls. This report describes an animal model of behavioral dysfunction in autoimmune disease. We have recently observed impaired exploration of a novel object in the MRL-lpr group. Moreover, this behavioral deficit significantly correlated with high serum autoantibody titers (a reliable symptom of autoimmunity). In the present study we test the relationship between exploratory behavior and autoimmunity by treating young MRL-lpr and MRL lpr/+ mice for 6 weeks with immunosuppressive drug, cyclophosphamide (100 mg/kg/week, i.p.). It was expected that such a treatment would prevent development of autoimmune symptoms and restore normal exploratory behavior in MRL-lpr mice. Indeed, chronic immunosuppressive treatment almost completely abolished the onset of autoimmune disease and increased the duration of novel object exploration in the MRL-lpr group. Thus, in MRL-lpr mice there may be a causal relationship between autoimmune factor(s) and impaired exploration of novel objects. (IBS is a OMH Postdoctorate Fellow; supported by funds from NERSC)
389.15 INTERLEUKIN-1 INHIBITS SEXUAL BEHAVIOR OF FEMALE BUT NOT MALE RATS. B. Yirmiya*, R. Avissar, O. Donchin, and E. Cohen; Department of Psychology, Bar-Ilan University of Jerusalem, Mount Scopus, Jerusalem 91505, Israel.

In response to infection and injury, a variety of cells release the cytokine interleukin-1 (IL-1), which produces immunologic and neuroendocrine behavioral effects. The present study examined the hypothesis that the previously reported suppressive effects of IL-1 on reproductive hormones and goal-directed activity are also associated with decreased inhibition of sexual behavior. The effects of IL-1 on sexual motivation and sexual receptivity in female rats were examined using the partner preference (PP) paradigm and the last of lusotus questionnaire. The effects of peripheral (i.p.) administration of IL-1 were examined after the sexual phase of intact cycling females. Administration of IL-1, at a dose of either 2 or 10 μg/kg, significantly decreased copulation (soliciting) and receptive behaviors. The suppression of sexual behavorination was further demonstrated in ovariectomized rats, following hormonal induction of estrus: IL-1 (2 μg/kg) injected 2 hr before testing significantly decreased PP scores as well as copulatory and receptive behaviors. Intracerebroventricular administration of IL-1 (10 ng/rat) suppressed PP scores and copulatory behavior in intact cycling females. The effects of IL-1 on male sexual motivation and performance were assessed using the PP paradigm and by counting the number of mounts, intromissions and ejaculations during a 15 min testing period. IL-1 had no effect on any component of male sexual behavior. The inhibition of female sexual behavior by IL-1 may be an adaptive mechanism, which prevents conception during an infection and therefore reduces the risk of prenatal infection.

Supported by the Volkswagen Foundation and the Israel Foundation Trustees.


Interferons are known to be antiviral and are up-regulated in lupus-like autoimmune diseases such as that seen in NZB x NZW F1 (B/W) hybrid mice. This study focused on the role of interferon-alpha (IFN-α) in the behavioral abnormalities seen in these mice by examining brain from female B/W mice for IFN-α mRNA. Using reverse transcription followed by polymerase chain reaction amplification (RT-PCR), IFN-α was detected in brains both before and after the onset of severe immunologic abnormalities (12 and 24 weeks). In situ RT-PCR was used to localize specific brain regions producing IFN-α. Following fixation and paraffin embedding, brains of 12 week old mice were sectioned in the sagittal plane, cryoprepared to permeabilize membranes and DNAase to destroy genomic DNA. mRNA was converted to cDNA in an RT reaction and IFN-α cDNA was amplified by PCR. A digoxigenin-labelled nucleotide was incorporated into the amplified product and detected immunocytochemically. Negative and positive controls were run with each sample. Positive signal for IFN-α mRNA was found in middle layers of the cerebral cortex, and extending along most of the anterior-posterior axis. Signal was seen in membranes surrounding the brain and in occasional cells throughout the CNS. Dysmorphology of the cerebellum was also apparent. To determine the specificity of this pattern of signal, brains of mice injected with the IFN inducer Poly IC were examined. These mice displayed IFN-α mRNA signal in neurons throughout the brain. Thus, it appears that the in situ RT-PCR technique is sensitive and specific. The production of IFN-α mRNA in the CNS may explain some of the behavioral alterations in autoimmune mice. Supported by MH49043, MH10643, MH15442 and HD04024 and by the Developmental Psychology Endowment fund of the Department of Psychiatry.


Stress is a complex phenomenon involving a highly coordinated activation of the endocrine, immune and central nervous systems. We have reported the central pattern of c-fos expression induced by intermittent footshock and the activation of central c-fos expression system suggests that the psychological dimension of footshock may be an important component in stressor c-fos expression. We have assessed the ability of conditioned fear to induce c-fos expression in the brain and to alter immune activity in male, B6D2F1 rats. Rats were given three sessions of intermittent footshock (UCS) with a tone as the conditioning stimulus (CS). A week later, animals were either re-exposed to the CS (cCS) and immediately sacrificed or not exposed to the tone (CS-). We found that footshock induced c-fos expression can be classically conditioned and that the cCS animals were immunosuppressed, relative to the CS- group. The conditioned expression occurred in the same brain regions observed following the UCS, whereas the cCS group showed minimal brain c-fos expression. Conditioned immune effects were also found in that the cCS group had significantly enhanced splenic NK cell activity, relative to CS animals. Splenect and blood cell lymphoproliferative responses to mitogen were also elevated in the cCS, relative to the CS group, thus conditioned fear can induce c-fos expression in a distinct central circuit as well as produce an immunomodulation. Presently, we are investigating the relationship between the strength of the conditioned response and the immune alterations observed during c-fos expression and the concomitant effects of conditioned fear on central c-fos expression and immune cell activity. Supported by MRC of Canada.


Interleukin (IL)-2 potentiates a variety of immune responses and influences cerebral nervous system activity. We reported that IL-2 induced expression of central monoamine activity that were similar to those provoked by uncontrollable stressors and that IL-2-induced immunomodulatory mechanisms were mediated by hypothalamic-pituitary-adrenal (HPA) systems. In the present study, we determined whether IL-2 would interact with a stressor to alter neuroendocrine and immune activity. BALB/c mice received either Ringer's Solution or IL-2 (200 ng, i.p.) and were immediately exposed to a novel environment for 0, 30 or 60 minutes. Seven fold elevations of plasma corticosterone concentrations were evident in IL-2 treated mice exposed to the stressor for 50 or 60 minutes compared with those measured immediately after IL-2 injection. These elevations were significantly greater than those observed in vehicle-treated animals exposed to the stressor for comparable periods. Corticosterone levels did not differ among vehicle and IL-2 treated mice that were returned to their home cages for 0, 30 or 60 minutes following injection. IL-2 also potentiated corticosterone elevations following exposure to restraint stress. Mice received vehicle or IL-2 were either not stressed or were immediately restrained for 30 minutes. Corticosterone levels were determined 0, 30 or 60 minutes after stressor termination. In vehicle-treated mice, levels were significantly increased immediately following restraint but returned to control levels within 30 minutes of stressor termination. In IL-2 treated mice, corticosterone levels were markedly elevated 0 and 30 minutes after stressor termination and returned to non-stress levels within 60 minutes of stressor termination. Additionally, the enhancing effects of IL-2 on an antigen-specific IgM PFC response were not evident in mice exposed to a mild stressor immediately after IL-2 administration. This treatment also resulted in a suppression of splenic T Cell proliferation and IL-2 production that was similar in magnitude and duration to that seen in mice exposed to restraint stress. It is suggested that neuroendocrine and immune consequences of a lymphokine (i.e. IL-2) are influenced by the stressor background on which it is superimposed. Supported by NIMH, MRC of Canada.

389.20 TO THE MECHANISMS OF PRENATAL EPYGENIC TRANSFER OF MATERNAL "ANTI-BRAIN" AUTOIMMUNE. N.K. Yabl- 

THEMECHIYCH,* A.R. Polataiev. Chernobyl-Test Ctr., Mosco-

cow, Russia.

Transfer of the specific autoimmune reactions from a mother, to her child (described earlier) is probably connected with an appearance of some effects of the nervous system. It is necessary to know the mechanisms that allow maternal immune system to play a "matrix" role in the immune peculiarities of a child. It can be supposed that the modulation of the maternal autoimmunity of female offspring of child lymphocytes (LC) by the maternal AB: (2) the "transplacental" activation of the immune system of child lymphocytes (LC) expressing autoantigens (autoimmune phenomena); (3) the maternal transmission of the immunosuppressive effect of the maternal LC (including memory cells); (4) the transfer of the maternal maternalization of the long (for years) persistence in a child. In expansion of this hypothesis (passively or actively immunized by the S100 proteins during pregnancy), the "mirror" phenomenon of the maternal AB is one of the explanations for the hypothesis (1) is groundless. In opposite real evidences for hypotumia (2) and (3) were receiv-

ed. Thus, the theory is probably based on the combination of the maternal maternalization of the child's autoimmune mechanisms and a direct transfer of the maternal maternalization of the LC with their subsequent persistence in a child.

Several lines of evidence indicate that surgical sterilization for breast cancer during the first half of their menstrual cycle show a 3-fold increase in metastatic growth. We present evidence in rats in support of the hypothesis that this phenomenon is attributable to metabolic development that is induced by the surgical procedure. The effect is also illustrated by elevated estradiol/low progesterone levels at the time of surgery. Suppression of innate immunity is suggested to underlie the increased susceptibility to metastasis, rather than a direct effect of sex hormones on the tumor. Syngeneic mammary tumor cells (MB16106) were injected intravenously into pre- and post-PH (control) rats during three different phases of the estrous cycle. The metaphase efficiency of this tumor, which is confirmed here to be highly controlled by natural killer (NK) cell activity, was higher during estrus phases that are hormonally homologous to the high-risk periods in women. Estrogen treatment alone caused similar effects in ovariolesteromized rats and progesterone partially blocked these estradiol effects, whereas neither hormone affected the proliferation rates of the tumor in vivo. The tumoral activity per blood NK cell was diminished during the high-risk estrous phases. These findings indicate an animal model for further studying the clinical phenomenon and suggest hormonal and immunological mechanisms mediating it. Supported by NIH-HDD7228, NIH/NS 07628, VA Medical Research Service, the UCLA Psychoneuroimmunology Task Force, and a research grant from Tel-Aviv University.

SEXUALLY DIMORPHIC EFFECT OF PRENATAL DHEA TREATMENT ON T-CELL FUNCTION. S. G. Shiota, I. Haras and F. Reda* Dept. of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104

We have hypothesized that sexual differentiation of the immune function occurs preferentially and is driven by similar events as seen in the sexual differentiation of the brain. Therefore, pregnant dams were treated with different doses of dehydroepiandrosterone (DHEA), a weak androgen, 100 μg/kg drinking water from day 8 of gestation until parturition when DHEA treatment was discontinued. Body weights of young, prepubertal (day 35 of age) male and female pups from the DHEA groups were significantly lower than those of controls, and showed a dose response effect even at this age (control males 133.6±2.6 g; DHEA males (250 ng/ml): 97.4±5.5 g; DHEA males (250 ng/ml): 93.2±2.2 g; DHEA males (250 ng/ml): 114.3±7.5 g; control females: 253.0±25.7 g; DHEA females: 26.2±26.6 g; DHEA females (250 ng/ml): 103.6±30.6 g). In contrast, both absolute thymic weights and thymic weights normalized to body weight were significantly higher in the offspring of the DHEA females (250 ng/ml) compared to those of individual animals representing different litters. There was no significant sex difference in the proliferative response to Con A in the control animals, although control males showed a tendency toward a higher response. Prenatal treatment with DHEA (2.5 mg/ml) significantly reduced proliferation in the male offspring (control: 210,468±53,357 cpm; DHEA: 90,897±2,594 cpm) while DHEA females were not affected significantly and showed an increased response than a diminished response (controls: 150,682±28,818 cpm; DHEA: 220,868±1,21,145 cpm). This data suggest that prenatal DHEA treatment permanently alters thymic function and T-cell dependent functions, particularly in the male offspring.


Glucocorticoids (GCs) are widely used in therapy for their antiinflammatory and immunosuppressive properties but their use in cytokine-related pathologies needs a thorough understanding of their role in modulating cytokine actions. The present study was designed to clarify the effect of GCs on the action of IL-1β on the serotonergic system. Changes in serotonin metabolism (hydroxyindoleacetic acid [SHIAA]) were recorded in freely moving rats by in vivo voltammetry using chronically implanted carbon fiber electrodes in the medial preoptic area (MPA) in the presence of 50 ng/kg DEX (40 μg/ml) induced an increase in 5 hydroxyindoleacetic acid (5-HIAA) levels; a rapid-short, term rise was followed by a lasting increase possibly due to newly synthesized IL-1. The synthetic GC dexamethasone (DEX) increased 5-HIAA levels in MPAs (50 mg/kg sc, 50 min before IL-1β) prevented the effect of IL-1β starting from 150 min, suggesting that it only inhibited the second increase. In adrenalectomized rats IL-1β had no effect but when pretreated with DEX (40 μg/ml) the effect of IL-1β was restored. The GC receptor antagonist RU38486 (25 mg/kg sc, 60 min before IL-1β) completely prevented IL-1β activation of the serotoninergic system. The results indicate that, although GC is effective inhibitors of IL-1 synthesis, their presence is necessary for IL-1β-induced activation of the serotonergic system. Thus GC may allow a number of corticosteroid-mediated actions triggered by IL-1 in the course of inflammation.


We have previously demonstrated significant and selective changes in numbers and percentages of lymphoid subpopulations and leukocyte subpopulations in the rat. These changes were rapidly induced by mild acute stress (2 h restraint), and consisted of a decrease in numbers and percentages of lymphocytes, and an increase in numbers and percentages of neutrophils. B cells, NK cells, and monocytes showed greater stress-induced decrease than T cells. Helper T cell number showed the lowest magnitude of decrease with stress. These stress-induced changes were rapidly reversed upon the cessation of stress.

In the present series of studies we demonstrate that adrenalectomy significantly reduced the magnitude of the stress-induced changes. Furthermore, administration of glucocorticosterone sodium phosphate inhibitor, eliminated the stress-induced changes in PBL. However, catecholamine receptor antagonists (phenolamine [alpha antagonist] and propranolol [beta antagonist]), failed to inhibit the stress-induced changes in PBL. Acute administration of corticosterone to adrenalectomized animals resulted in a close replication of the stress-induced changes observed in adrenal intact animals. Furthermore, acute administration of glucocorticosterone receptor agonists showed that the Type II receptor agonist (RU28362) replicated all the stress-induced changes in PBL (except the decrease in NK cell percentage). The Type I receptor agonist (aldosterone) had no effect. These results suggest that corticosterone is a major mediator of the stress-induced changes in leukocyte distribution. (MH 47674, MacArthur Foundation.)

TYPE II GLUCOCORTICOID RECEPTOR (GR) GENE EXPRESSION PARTICIPATES IN THE DEVELOPMENT OF NEUROENDOCRINE-IMMUNOLOGICAL SEXUAL DIMORPHISM: EFFECT OF ABNORMAL GR FUNCTION IN TRANSGENIC ANIMALS BEARING TYPE II GR ANTISENSE RNA. B. Marchetti*, M.C. Morais and N. Baden. Dept. of Pharmacology, University of Catania, Italy, and Molecular Psychogenetics, CHUL, Quebec, Canada.

Clinical and experimental evidence indicates that gonadal steroids can modulate immunological functions and that a sexual dimorphism exists in the immune response to different xenobiotics in the male and female, however, involved in sex-dependent immune responses are not completely understood. We have recently (Endocrine Journal 2:181, 1993) demonstrated that type II GR gene expression within the thymus is markedly modulated by physiological alterations of the sex steroid hormone milieu. The aim of the present study was to determine the role of type II GR gene expression in the development of neuroendocrine-immunological sexual dimorphism using a transgenic animal model created by partially knocking out gene expression with type II GR antisense RNA. In intact mice, a sexual dimorphism in the post-natal development of type II GR gene expression paralleled the development of sexually dimorphic immune responses, initiating 9 days after birth, reaching a peak of activity at 25 days of age, and declining around puberty. The development of a sex-dependent apoptotic potential after thymocyte treatment in vitro with corticosterone followed a similar pattern. In transgenic animals, the abolishment of a sexual dimorphism of type II GR gene expression was accompanied by a lack of sexually dimorphic immune responses and marked inhibition of the specific suicide program within the thymus, suggesting a key role for type II GR gene expression in the molecular mechanisms underlying the development of sexual dimorphism of immune responses.


The hypothalamus (HYF) and thymus gland (THY) both contain bio- and immunoreactive CRF and CRF mRNA (Redei, 1992; 1993). Hypothalamic secretion of CRF is a well-established final common pathway for direct and indirect immunomodulation, but the role of CRF in THY function is not well understood. We have compared the in vivo release of CRF during a 6 hr perfusion of preoptic area-medial hypothalamic (POA-MH) HYF and the THY, individual POA-MBH and THY were removed from male rats (350-450g) and placed in parallel perfusion chambers containing Earls’ balanced salt solution (pH 7.4) at 37°C. POA-MBH and POA-MBH+HYF were perfused at a rate of 2 μl/min, while the rate of the lobe of the thymus was lowered to 0.2 μl/min. After a 60 min preincubation period, fractions were collected (500x10/10 min) collected over 2 hr for basal release of CRF assessed by RIA (kg 6 g/ml) and after CRF was increased in the medium for a third time during the 90 min. At the end of the incubation each POA-MBH and THY was weighed and assessed for total CRF. As expected there was an 18 fold higher CRF secretion in HYF compared to THY. However, an injection of total secretion using a numerical integration of the release curve (trapezoidal approximation) revealed that total THY secretion was approximately 10 times greater than POA-MBH+HYF. Experimental lesions of the HYF which resulted in THY inhibition and subsequent increased response to inulin stimulus did not result in significant changes in CRF secretion by the thymus. Supported by NIH MH46808.

Numerous studies have reported the production of a variety of neuropeptides or peptide hormones by the immune system. CRF appears to play a role in cell proliferation, differentiation, and function. Recent studies indicate that CRF may also be involved in the regulation of the immune system. In this study, we have investigated the localization of CRF in the primary and secondary lymphatic organs of the rat. We have found that CRF is present in all the lymphatic organs studied, including the thymus, spleen, and lymph nodes. The results of this study suggest that CRF may play a role in the regulation of the immune system.

390.8 IMMUNE AND ENDOCRINE IMPLICATIONS OF LONG-TERM INTRACEREBROVENTRICULAR CORTICOTROPIN-RELEASING HORMONE ADMINISTRATION IN THE RAT. J.H.M. Reul*, M.S. Laberot, G.J. Wingers, E. Arzt and E. Holzbecher, Max Planck Institute of Psychiatry, Clinical Institute, Department of Neuroendocrinology, Munich, FRG.

The brain, endocrine and immune systems communicate as an integrative network, which is critical for maintaining homeostasis during disease and other stressful situations. To investigate the physiological consequences of chronic hypophalmic-pituitary-adrenocortical (HPA) axis hyperactivity, rats were injected with corticotropin-releasing factor (CRF) for 1 week with CRH via an osmotic minipump and HPA axis and immune system function were studied. As compared to the vehicle, CRH produced elevated a.m. plasma ACTH and corticosterone levels, increased anterior pituitary POMC mRNA expression, thymus involution and adrenal enlargement. Regarding immune function, CRH treatment had markedly different effects on splenocyte proliferation and cytokine expression. Long-term CRH-created rat or rat-derived cells were examined for new responses in situ. Histologically, lymph node and thymus sections revealed no obvious morphological changes. However, chronic CRH exposure resulted in a marked increase in CD4+ and CD8+ T cells in the spleen and thymus. These results suggest that chronic CRH exposure may contribute to the development of chronic immune dysfunction and may have long-term implications for the maintenance of homeostasis.
391.1

EFFECT OF BACTERIAL ENDOTOXIN ON HIPPOCAMPAL AND PREDICTIVE NEUROTRANSMISSION, BODY TEMPERATURE, BEHAVIORAL ACTIVITY AND FREE CORTICOSTERONE LEVELS. A.C.E. Lindorfer*, C. Flachkampf, F. Holzhofer and J.M.H.M. Reul, Max Planck Institute of Psychiatry, Clinical Institute, Department of Neuroendocrinology, Munich, FRG.

Evidence has accumulated for a bidirectional communication between the central nervous system and the immune system. However, on the level of the brain the mechanisms involved in the processing of signals from the immune system largely remains to be resolved. We started a study on the effects of i.p. lipopolysaccharide (LPS, bacterial endotoxin) administration on hippocampal and preoptic serotonergic and noradrenergic neurotransmission in freely moving rats. In addition, we monitored hypothalamic-pituitary-adrenocortical (HPA) axis activity by measurement of free corticosterone levels in the dialytes. Behavioral activity was scored by measuring the time during which rats were active and body temperature was assessed via biotelemetry. I.p. LPS treatment produced a dose-dependent increase in the extracellular concentrations of serotonin in the hippocampus but not in the preoptic region. In addition, the endotoxin caused a dramatic decrease in preoptic levels of noradrenaline, elevations in body temperature and HPA activity, and a decrease in behavioral activity. We conclude that signals from the immune system exert different effects on brain neurotransmission. These distinct changes in neurotransmitter may be involved in the regulation of specific neuroendocrine, autonomic and behavioral responses after an immune challenge.

(VW grant 1/68 430)

391.2


It has been shown that injection of lipopolysaccharide (LPS), a powerful bacterial endotoxin, results in a dose- and time-dependent expression of the proto-oncogene c-fos in the brain (Wan et al, B.B.K. 1:25; 51993). We have extended these studies and report here on the transmitter specificity of hypothalamic neurons that are activated following LPS injection.

LPS (100 µg) was administered i.v. to male 5-6 d rats. Two hours later the rats were perfused and the brains processed for c-fos immunocytochemistry (rabbit anti-fos, Santa Cruz). Immunocytochemical and histochemical methods showed that many of the c-fos positive neurons were also positive for tyrosine, vasopressin and NADPH-dihydrosaprase (NADPH-d). Double labelled cells were predominantly located in the paraventricular and supraoptic nuclei of the hypothalamus and analysis showed that NADPH-d > OXY = AVP in numbers of c-fos co-localized. Raw cell counts showed that LPS decreased NADPH-d staining, increased OXY, and had no effect on AVP. The LPS-induced changes in NADPH-d suggested a functional role for nitric oxide (NO). Consistent with this finding, preliminary results indicate that inhibition of NO synthase by i.c.v. injection of L-NAME potentiates the induction of c-fos protein by LPS. Supported by KBC and NIMH.

391.3


Centrally administered IL-1 results in a large increase in serum IL-6. However, the role of the brain's role in this response is yet to be characterized. The mechanisms of this induction and the signal conveying the information from the brain to the periphery are not known yet. To help characterize IL-6 pathway of IL-6 induction, IL-6 levels, measured as hybridoma growth factor on 7TD1 cell line, were evaluated in rat serum and cerebrospinal fluid (CSF) at different times after intracerebroventricular endotoxin (LPS, 2.5 µg/rat). In the same experiments, IL-6 mRNA expression, measured by Northern blot analysis, was evaluated in peripheral (in adenals and lymphocytes) and in the brain (in hypothalamus, hippocampus and striatum). In serum, the highest IL-6 levels were reached at t = 2 h after which they rapidly decreased. In the same time-course was showed in IL-6 mRNA in adenals and lymphocytes. A different pattern was present in the central nervous system: the CSF, IL-6 was detectable starting from t = 2 h, reached a plateau at t = 4-8 h and remained detectable until t = 16 h. IL-6 mRNA expression in the brain areas showed a similar time-course, reaching a maximum at t = 4-8 h. The results indicate that IL-6's synthesis is differently regulated in the brain and in the periphery.

391.4

QUINOLIC ACID IMMUNOREACTIVE CELLS IN THE BRAIN AFTER INTRACEREBRAL INJECTION OF LIPOPOLYSACCHARIDE. M.G. Esposito, J.R. Nofflet and M.A. Hannabold*. Georgetown University, Biology Department, Washington, DC 20057-1028.

Quinolic acid (QUIN) is a neuropharmacological tissue metabolite. Antibodies to quinolic acid were used to study its cellular localization in the brain 1 to 30 days after intracerebral injection of lipopolysaccharide (LPS). Quinolic acid immunoreactive (QUIN-IR) cells were observed in numerous regions of the forebrain, corpus callosum, neocortex, meninges and choroid plexus 10h after LPS application. The immunoreactive neurons were larger than lymphocytes, ranged from round to round-shaped in control and increased their number and morphology, and were often observed around the vasculature. The overall number of QUIN-IR cells increased at 48h, and peaked in number at 72h. In saline injected control animals, only rare QUIN-IR cells were observed at 72h surrounding the injection site. The number of QUIN-IR cells was reduced in the peri-injection region by 4 days after LPS injection, but did not return to control levels until 15 to 30 days later. Tissue destruction was observed in the cortex, hippocampus and corpus callosum, and progressed in severity during the days one and four. No clear correlation with QUIN-IR was found with the monoclonal antibodies EN1, ED1, F480, OX34 or OX41. The tissue damage observed around the injection site correlated more closely with a central mass of infiltrating leukocytes than with the separately scattered QUIN-IR cells observed in and around the damaged region. These results suggest that QUIN, derived from a select population of leukocytes, may contribute to some of the secondary neuronal death subsequent to CNS infections.

We propose that QUIN, rather than being simply a neurotrophic byproduct of tryptophan metabolism, may be a cytokine or immune modulator involved in the initial reactions to pathogens.

391.5

PROHORMONE CONVERTASES PC2 AND PC1 IN RAT NEUTROPHILS AND MACROPHAGES. A. M. Ballabio, C. Chabot*, C. Cottier, J. A. Spoiler* and E. B. Espinoza*. Dept. of Biochem. and Mol. Biol., Dept. of Physiology and Dept. of Med./Rheumatology. LSU Medical Center, New Orleans, LA 70112.

Prohormone- or proencephalin-converting enzymes PC2 and PC1 have been observed exclusively in cortical and subcortical regions. While the presence of these enzymes in cells of the immune system was demonstrated. PC2 was detected in peripheral and liver-infiltrated polymorphonuclear leukocytes (PMN) but not in alveolar macrophages (AM) or peritoneal mononuclear cells (SMC). PC2 is expressed by circulating PMN in vivo and in vitro but not in PMN, and a 66 kDa protein was the only PC1 form detected. Proenkephalin-derived peptides (PEN2) were observed in PMN. AM, and SMC by peptidase assay. 33, 25, 18, 21, 14 and 13 kDa in the liver cells and a doublet of 35 and 32 kDa in the livers. PC2 and PEN2 increased in liver PMN and peripheral PMN 90 min after intravenous (i.v.) infusion of LPS, suggesting an increased expression. However, in vitro assays showed that the proenkephalin peptide FMRF was not increased the basal secretion of PC2 proteins and PEN2 in PMN. These results indicate that PC2 proteins are released from PMN, together with PEN2, and that LPS in vivo may act through an indirect mechanism. Low levels of PC3 and PEN2 were detected in the am of rats treated for 90 min with SAL or LPS. However, a significant increase of PC3 and PEN2 appeared 30 h after LPS infusion. These results show for the first time that PC2 and PEN2 are differently expressed in PMN and AM, respectively, which were paralleled by the presence of different post-translation products of PENK. In addition, the in vivo effect of LPS on PC2, PEN2 and PEN5 levels in PMN and AM revealed the effect of LPS on proenkephalin levels in endocrine tissues, suggesting that similar mechanisms may control the turnover of PENK in neuroendocrine and in immune cells.

391.6


Pikriyova, Jilek and Winiwarter (CRC Press, 1992) described a very comprehensive mathematical model of the immune response to antigen. This model consists of 12 simultaneous first order differential equations and 8 auxiliary equations ("switches"). Eight cell types and 4 molecular messengers are represented. Here we describe an extension of the Pikriyova model which includes for NEI modulation. These equations are derived from our experimental investigation of the adrenal axis response to variable in doses of endotoxin (EN, E, C) to 50 and 100 µg/kg per b.w. administered to male SD rats (50-600 g) 30 min after IP injection of endotoxin. Rats received either saline or EN at the indicated doses and blood was withdrawn at 0, 0.5, 1 and 4 hrs later. Plasma ACTH and corticosterone (C) was assayed by RIA. Relative increase of EN resulted in parallel increased release of B at all intervals. However, ACTH release after 10 µg EN resulted in the greatest (2000) increment from time 0 when compared to other EN treatments. The maximum was only a 3X increase, whereas 50µg resulted in a 10X increase. Inverse changes in ACTH after EN may be explained at the N-P level, whereas the B response may be a direct cytokine effect that controls ACTH release. Overall, the mathematical model of the NEI Axis integrates these and other findings from our experiments to stimulate NEI regulation. Supported by NIH MH48608.

It is generally believed that during a viral infection, cytokines stimulate the release of endogenous adrenal steroids which in turn can feedback inhibit stress on evolving immune responses. Although ample data demonstrates that cytokines stimulate HPA axis activity, few studies have directly examined adrenal steroid secretion during viral infection. Accordingly, we examined steroid secretion at multiple time points in the am and pm of mice infected with lymphocytic choriomeningitis virus (LCMV). Since our previous studies have indicated that adrenal steroid secretion levels are similar between infected and control animals, we examined corticosterone levels at the receptor level, we also measured cytotoxic type II adrenal steroid (glucocorticoid) receptor binding in immune tissues. LCMV-infected mice exhibited modest elevations in corticosterone secretion on Days 3 through 7 post infection. In corticosterone values were greater than am values in all cases. Cytotoxic type II receptor binding in spleen and thymus of LCMV-infected animals was significantly decreased in the pm compared to non-infected animals, indicating that infection-induced increases in corticosterone in the pm were activating receptors in these tissues. Interestingly, however, significant decreases in cytotoxic type II receptors were also observed in the am of spleen of infected mice. These receptor decreases occurred in the absence of marked elevations in corticosterone and suggest that local factors (eg cytokines) elaborated during the immune response to LCMV may directly influence adrenal steroid receptor expression. Our studies on the interferon inducer, poly I:C, indicate that interferons may mediate these adrenal steroid-immune interactions in LCMV infection by augmenting corticosterone production and receptor expression in the presence or absence of hormone. Supported by MH41764 and MacArthur Foundation.

391.8 IMMUNE CELLS MEDIATE CHROMAFFIN AND SYMPATHETIC GANGLION CELL CATECHOLAMINE SECRETION. S.Jones, R. Litwin, J. Pintner1, J. Roberts, J. Walter1, and P. DaPoletto. Loyola Med. Center and Hines VA hospital, Maywood IL, Univ. Health Sci./The Chicago Med School (Mt. Sinai Hospital USA and Neuropharmacology, Univ Antwerp, Belgium).

Cytokines, such as tumour necrosis factor (TNF) and interferon-gamma (IFN-gamma) are secreted by activated T cells in response to mononuclear cell conditioned media (Life Sci.33,447-451) suggests that immune cells may mediate stress hormone secretion. In the present study, porcine chromaffin (C) and ganglion (G) cells were used to test the possibility that porcine cytokines and bovine immune cells could stimulate secretion. Blood, spleen, adrenal and superior cervical ganglion were obtained from local slaughter houses. Mononuclear cells isolated from blood or spleen were incubated (37°C) overnight in RPMI media without serum; cells were removed and conditioned media (CM) frozen and kept at -20°C. Purified C and G cells were isolated using collagenase and maintained in culture at 37°C. Secretion experiments were performed with 5:7-days for C (cultured in DMEM/F12, 10% FBS) and at 4 days for G (cultured in F12, 5% Horse serum). For secretion experiments, growth media was replaced with CM or control RPMI for 90 min, at 37°C. For C, Epiphrine released into conditioned media was measured by HPLC while G secretion involved assessment of preformed 5HT. Secrecy in response to CM is expressed as % of total cell content.


Recently we demonstrated that primary immunization with sheep red blood cells (SRBC) decreases hypothalamic and cortical serotonin (5-HT) levels in F344 rats while increasing extracellular 5-HT levels (Dardier et al., Brain Res., 1994). Pretreatment with an immunosuppressive drug, cyclophosphamine prevented these effects suggesting that a T lymphocyte product, but not a macrophagic one, may be involved in this serotonergic activation induced by SRBC. In the interleukin-6 (-IL-6) drug might be a good candidate for being a link between the immune system and the serotoninergic system. To characterize further this hypothesis, we have investigated the effects of keyhole limpet hemocyanin (KLH, 200 µg i.p.) on both hypothalamic IL-6 levels and the hypothalamic-pituitary-adrenal (HPA) response (plasma ACTH and corticosterone concentrations). The presence of specific antibodies for KLH (IgM) in the serum was also assayed by using an ELISA method at 3, 4 and 5 days following KLH injection. The hypothalamic IL-6 level significantly decreased by -26% (p<0.05) while IL-6A-IIA levels increased by +43% (p<0.01) while IL-6 levels increased as compared to controls 4 days following the primary immunologic response to KLH. In the cortex and hippocampus, no changes were observed in the serotonergic markers studied. Antibodies directed to KLH were present since Day 3 following KLH injection. Plasma ACTH levels in KLH treated rats were higher than responses in controls at all points, while corticosterone levels were peaked at Day 4 post-immunization. This data confirms our previous report showing that long-lasting changes occur in the hypothalamus as well as in the HPA axis when the production of specific antibodies is maximal following primary immunization.

391.10 N-2-HYDROXYETHYL)HEXADECANAMIDE IMPROVES NEUROLOGICAL DEFICITS ACCOMPANYING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN RATS. A. Lom1, R. Carrella, and S. Mazzari. Researche S.C.P.A., Centro di Ricerca Biomedica, Ospedale Civile, 31033 Castelfranco Veneto, Italy.

Mac cells (MCS) present in the normal CNS have been proposed to regulate interactions between the immune and nervous systems. In addition, it has been suggested that MCS are involved in the pathogenesis of experimental allergic encephalomyelitis (EAE). Recently, the endogenous N-Acylamide N-2-hydroxyethyl)hexadecanamide, has been proposed to behave as a local autacoid or a mediator capable of negatively modulating CNS activation. In agreement with this concept, treatment of the animals (ALX) with N-2-hydroxyethyl)hexadecanamide (LG 21101) prevents MC-dependent edema formation and substance P-induced MC activation in the mouse ear pinna (Mazzari et al., this meeting). Here we have evaluated the effect of MC modulation by LG 21101 in the development/progression of EAE in B10.PL mice after immunization with I-A-n Cad, the Thy1.1-tergulated, acetylated peptide of mouse myelin basic protein (Rajewski et al., Science 250, 1213, 199). After treatment with LG 4.0 (10 mg/kg) given beginning on the day of immunization delayed the onset (6 day post-immunization) as well as the frequency of animals displaying the neurological syndrome. In addition, when treated was begun on day 4 post-immunization (F1), LG 21101 significantly reduced the severity of EAE. The exogenous N-Acylamide alleviated the clinical scores at day 8 P.I. up to day 31 P.I. (p< 0.05 versus vehicle, n = 19-20). These findings not only support a physiological role for N-2-hydroxyethyl)hexadecanamide, but also suggest that this type of molecule may represent a novel therapeutic approach to the management of inflammatory conditions of the nervous system modulated by MCS, including autoimmune CNS diseases.

391.11 THE CD4 CORECEPTOR MEDIATES EFFICACY OF T CELL ANTIGEN RECEPTOR INTERACTIONS WITH AUTOLOGOUS MYELIN BASIC PROTEIN. M.D. Mannpie1 and G. White. Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville NC 27834-4534.

Guinea pig (GP) myelin basic protein (MBP) is approximately 100-fold more active than rat (R) MBP as measured by induction of experimental autoimmune encephalomyelitis (EAE) in Lewis rats. A Ser for Thr substitution at position 80 comprises the distinguishing structural difference between the antigenic regions of GMPB and RMBP, respectively. To test whether the CD4 co-receptor has a differential role in T cell responses to these two proteins, we measured the inhibitory effect of the anti CD4 antibody W3/25 on in-vitro proliferative responses of MBP-specific T cell lines. W3/25 only partially inhibited proliferative responses to GMPB but fully inhibited the response to RMBP. This differential susceptibility to the inhibitory effects of W3/25 was observed across broad concentration range of antibody and antigen as well as across a broad range of T cell densities. T cell subpopulations or downregulating the CD4 expressed responses to GMPB but did not respond to RMBP. These results suggest that the response to autologous RMBP, unlike GMPB, is completely dependent upon CD4. In addition, T cell proliferative responses to GMPB that were obtained in the presence of W3/25 were fully antagonized in a concentration dependent manner by RMBP. These results indicate that MBP is a specific antigenic when CD4 is neutralized by an anti-CD4 monoclonal antibody. This work was supported by a grant from the National Multiple Sclerosis Society.


In situ hybridization of 35S-labeled cRNA probes was used to investigate the distribution of cDNAs encoding human p60 and p80 subtypes of the tumor necrosis factor receptor (TNF-R) in normal and herpes simplex virus (HSV) infected trigeminal ganglia in the mouse. Riboprobes were derived from previously described human equivalent length cDNA species provided by Immunex Corporation. Trigeminal ganglia were infected via cerebrocortical inoculation using a McKrae strain of HSV three days prior to analysis. In situ hybridization produced a moderately intense autoradiographic signal over subgroups of neurons and neuroglial cells in both normal and HSV infected ganglia. In general, the signal intensity and the moderately characterized neuronal labeling within trigeminal ganglia did not change in response to acute HSV infection. However, infection was accompanied by an intense white blood cell infiltrate, and many of these non-resident cells did display signal for both TNF-R subtypes. Signal over control sections hybridized with sense p60 and p80 TNF-R cRNA was comparable to background. The presence of TNF-R mRNA over neurons and astrocytes in the HSV infected trigeminal ganglia suggests a role for TNF in normal trigeminal functioning, and supports studies implicating TNF in the pathogenesis of immune-mediated peripheral neuropathies.

Supported by a grant from Research to Prevent Blindness (T.P.M.) and grant # MH47680 (P.E.B.).
13.11 ISOLATION OF C1q PROTEIN FROM RAT BRAIN FOLLOWING INJURY
Departments of Pathological Sciences, Elzeo Andrus Gerontology Center, South Calif., Los Angeles, CA 90089.

There is increasing evidence for a role of complement (C) components in the neurodegenerative process. The presence of activated C is associated with senile plaques and neurofibrillary tangles (Ekenboom et al., 1992). In this laboratory, we have been developing a model of acute brain injury in the rat. Following trauma, we find a distinct metabolic activation of complement components in the injured brain (Johnson et al., 1992; Lampert-Echterns et al., 1993). Animal studies showed an increase in C3 in CRH after systemic calcium (Ca) or saline injections (Piasnetti et al., 1993; Johnson et al., 1992). Although CRH is not always accompanied by an increased translation of protein in brain or spinal cord, these complement studies provide evidence that CRH activates the complement system in brain. The present studies utilized protein chromatography and Western blots to identify CRH protein increases in brain after injury. Animal findings are consistent with the hypothesis that complement activation may be an important mediator of brain injury.

13.12 NEURAL-IMMUNE INTERACTIONS: RESPONSES TO IMMUNOLOGIC CHALLENGE
Department of Microbiology and Immunology, The University of Connecticut Health Center, Farmington, CT 06030.

The brain is considered an immunologically privileged site due to the blood-brain barrier (BBB). However, this barrier has been shown to be permeable to a variety of materials under certain conditions. The BBB may contribute to the development and progression of disease through the transport of effector molecules from the systemic circulation to the brain and vice versa. In this study, we investigated the effects of immune system activation on BBB permeability using the experimental autoimmune encephalomyelitis (EAE) model. EAE is an autoimmune disease of the CNS that is induced by injection of CNS antigens into susceptible animals. In this study, we used the Lewis rat model of EAE, which develops a chronic inflammatory response in the CNS after immunization with myelin basic protein (MBP).

13.13 RECRUITMENT OF MAST CELLS INTO THE CNS DURING DEVELOPMENT AND RESPONSE TO INFECTION IN THE MICE
Department of Neurology, Tufts University School of Medicine, Boston, MA 02111.

Mast cells are a type of innate immune cell that are distributed throughout the body and play a key role in inflammatory responses. In the central nervous system (CNS), mast cells are found in the meninges, blood-brain barrier (BBB), and peripheral nerves. Mast cell activation results in the release of histamine, tryptase, and other mediators that contribute to the inflammatory response. In this study, we investigated the recruitment of mast cells into the developing and adult CNS using intravital microscopy. We found that mast cells are recruited into the CNS during development and that their numbers increase following CNS injury. These findings have implications for understanding the role of mast cells in neurological diseases.

13.14 NERVE GROWTH FACTOR IMMUNOREACTIVE MAST CELLS IN HUMAN PERIPHERAL NERVE
Department of Neurology, Tufts University School of Medicine, Boston, MA 02111.

Mast cells are a type of innate immune cell that are distributed throughout the body and play a key role in inflammatory responses. In the central nervous system (CNS), mast cells are found in the meninges, blood-brain barrier (BBB), and peripheral nerves. Mast cell activation results in the release of histamine, tryptase, and other mediators that contribute to the inflammatory response. In this study, we investigated the recruitment of mast cells into the developing and adult CNS using intravital microscopy. We found that mast cells are recruited into the CNS during development and that their numbers increase following CNS injury. These findings have implications for understanding the role of mast cells in neurological diseases.
391.19

ACTIVATION OF THE BLOOD COMPLEMENT SYSTEM IN THE HUMAN SUBARACHNOID SPACE P.J. Lindsberg*, J. Oman, T. Lehto, M. Kaste, S. Meri, Departments of Neurology, Neurosurgery, and Bacteriology and Immunology, University of Helsinki, Helsinki Fin-00014, Finland.

The blood-brain barrier is deeply sectioned from the mammalian CNS by circulating immunological factors such as complement (C), a main mediator of humoral immunity and killing of foreign cells. C activation in plasma is under stringent regulation to prevent its attack on host cells. Since a number of CNS disease states induce plasma extravasation, which may carry C proteins to escape the regulation effective in plasma, we examined whether spontaneous C activation occurs in CSF in vivo and in vitro during subarachnoid hemorrhage (SAH). C activation on serum from healthy individuals was incubated at 37°C for 30 min with varying concentrations of serum before SCSB-9 concentrations were assayed with ELISA. In CSF, serum admixtures of 1:1 and 1:4, the assembly of Cl of SCSB-9 was up to five times enhanced by the addition of human CSF (n=5). CSF and plasma from SAH patients were studied on day 1 and 8 after SAH (n=15) and compared to controls with no CNS disease (n=8) and patients with ischemic stroke on day 1 (n=7). The SCSB-9 concentration during SAH on day 1; 2.61 ± 0.71 ng/ml was higher than that in plasma; 6.31 ± 1.7 ng/ml (p < 0.001), while no SCSB-9 was detected in the CSF of controls or patients with stroke (p < 0.001). The level of CSF SCSB-9 decreased during 8 days after SAH (24 ± 11 ng/ml). We conclude that CSF lacks C regulatory capacity, thereby permitting intrathecal C activation during SAH. C activation may promote chemotaxis, vasoactive perturbations and membrane lysis, but its potential pathophysiological role in SAH needs further studies.

391.20

BRAIN INFILTRATION BY AUTOANTIBOIES INDUCED BY CENTRAL INTERLEUKIN-2 (IL-2) INFUSION. U.-K. Harisch*1, J. Neubauer*, R. Quirion*2 and H. Kettenmann3.

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IL-2 regulates activities of immune and neural cells and has clinical application as an immunotherapeutic agent to treat peripheral and intracranial tumors. In this study, infusion of IL-2 (i.c.v., 5 U/hr for 14 days) in rats is shown to cause neuronal loss and periventricular damage around the infusion site, along with a pronounced activation of glial cells. Within the ventricle, masses of non-neural cells intermingled with collagen and fibronectin formed a vascularized pseudotumor clot that dislodged adjacent brain regions. By means of specific antibodies, immunocytochemistry revealed not only extravasating T cells, but B cells scattered throughout the clot as well as peri-ventricular tissue. At the ultrastructural level, the B cells were identified as plasma cells endowed in protein synthesis. Accordingly, a massive inundation by genuine immunoglobulin (lg) was detected in widespread brain areas. Fractions of the rat Ig apparently recognized populations of neural cells and astroglia. The results may thus be relevant both to CNS disorders involving an autoimmune component and to the adverse neurologic and neuropathological conditions induced by the application of IL-2 in the treatment of cancer. Supported by the BMFT (Germany).

392.1


Tumor necrosis factor-a (TNF) has been reported to have a variety of effects on neurons and neuroblastomas. It promotes axonal projection beyond the point of injury in severed optic nerve (Schwart et al., Brain Res., 1991, 545, 334), induces differentiation of neuroblastoma in conjunction with interferon-gamma (Ponzol et al., J. Cell. Biochem., 1992, 52, 3194), and protects neurons from metabolic-excitotoxic insult (Cheng et al., Neuro., 1994, 12, 139). Using the MTT viability assay, we have observed changes in differentiated and undifferentiated NIE-115 neuroblastoma cells (cytotoxicity indices [CI]) of 54±15% and 70±11 for differentiated and undifferentiated cells, respectively, treated with 250 U/ml TNF-a. TNF effects on differentiated NIE-1's with hTNF-a, an agonistic specific for the murine 55 kD TNF receptor, resulted in a CI of 79±2% at 200 U/ml TNF, indicating the likelihood that cytotoxicity in NIE's is mediated through the murine TNF receptor. Low TNF concentrations produce little or no cytotoxicity in NIE's. The CIs for differentiated and undifferentiated cells treated with 0.025 U/ml TNF-a are -11±4% and 16±17, respectively. In undifferentiated NIE's treated with recombinant human TNF-a, the CI is 70±16% (0.004 U/ml TNF-a). It is not clear whether the negative cytotoxicity index observed in differentiated NIE's at low TNF concentrations is due to an increase in cell proliferation or an increase in cell survival. The former seems unlikely, as the cells are treated with actinomycin D to inhibit proliferation during the course of TNF treatment. However, experiments measuring DNA-synthetic enzyme uptake are being performed to verify that proliferation is not occurring. It has also been reported that TNF is capable of promoting cell adhesion (Mackay et al., J. Exp Med 1993 177, 1277), so it is conceivable that TNF may promote the survival of differentiated NIE's by increasing their adhesion, either to substrate or to one another.

392.2

LOCUS COERULEUS NEURONS RESPOND DIFFERENTIALLY TO INTERLEUKIN-1B AND CORTICOTROPIN-RELEASING HORMONE. M.K. Homesty and J.M. Weiss. Neurosciences Program and Department of Psychiatry, Emory University School of Medicine, Atlanta GA, 30322.

We administered i.c.v. recombinant human interleukin-1B (IL-1B; 0.1-2.5ng) while recording extracellular activity of single LC neurons in Sprague-Dawley rats anesthetized with chloral hydrate or halothane. The finding that, in chloral hydrate-anesthetized rats, IL-1B dose-dependently suppresses LC firing rates. LC sensory-evoked responses, measured as the response to 1-second paw compression, were decreased by IL-1B proportionately with the baseline firing rate. In contrast to the effects seen in animals anesthetized with chloral hydrate, firing rates of LC units in halothane anesthetized rats did not change in response to any dose of IL-1B. Since corticotropin-releasing hormone (CRH) is an intermediary of IL-1 in many of the monkey's physiological actions, we compared the IL-1B responses of LC neurons to i.c.v. CRH (1-3ug) under halothane and chloral hydrate anesthetics. CRH did not change LC firing rates in rats anesthetized with chloral hydrate. As reported elsewhere (Valentino et al., Brain Res. 270: 363, 1983), LC units in halothane anesthetized rats were excited by CRH at both 1ug and 3ug doses. The magnitude of sensory-evoked responses under halothane was not affected by CRH, but since baseline firing rates increased, the relative size of the sensory-evoked response was decreased. Supported by NIMH grant MH54290.

392.3


Our laboratory has been studying the effects of interferon (IFN) on the hypothalamic pituitary adrenal axis (HPA). Last year we reported (Soc Neurosci Abstr. 1992:1900) that natural rat IFN alpha/beta (RIFNa/0) (La Jolla Molecular Research Lab) stimulated the HPA as determined by the RIA measurement of ACTH, betaendorphin and corticosterone (B) in plasma whereas human recombinant IFN alpha (hIFNalpha) (Hoffmann LaRoche) had a minimal effect. In this report we extend those conclusions to include the same animal preparation, e.g., 350-450 g Sprague-Dawley freely behaving male rats with indwelling jugular catheters. When either hIFNalpha or RIFNa/0 is administered i.v. at dose levels of either 200 or 600 international units (IU) per body weight, secretion of both ACTH and (B) is stimulated. Due to large variances, the differences in individual times between control (either saline, serum albumen or heat inactivated RIFNa/0 and IFN) injected animals were not always significant although mean differences were 2 to 4 fold. Total secretion of both ACTH and B is significantly increased by both IFN alpha although the response to RIFNa/0 was greater and with less variability as compared to hIFNalpha. Total secretion was estimated by numerically integrating the release curve (temporal profile), the amount integration being weighted by the secretion of ACTH after 600 IU being about half of that of 300 IU whereas there is no apparent reduction in B secretion. Supported in part by MH46808, DA 05723 and DA07978.

392.4


IL-1 and other cytokines are known to be an integral part of the acute phase response and the hypothalamic has been postulated to be site of interaction of cytokines, particularly with respect to regulating NE release in the brain. In previous experiments we had shown that cytokines including IL-1 probably do not act directly on the hypothalamus. In our current experiments we used microdialysis in an attempt to show that IL-1 does not act centrally but acts peripherally, using NE as index of measurement. We used microdialysis for our experiments and a specially constructed CMA-12/ Cunnami Combo. Animals used were Sprague Dawley rats weighing between 200-300 gms (Taconic Farms), Anaesthesia used was Chloral Hydrate. Basal values of NE were established 60 min post insertion of probe. At 140 min IL-1 was then infused through the cannula at a concentration of 100 ng / ml (total delivered was 5 ul over a 20 min period. Analysis of dialysate was done by HPLC with amperometric detection and samples were analyzed for norepinephrine. Averaged NE values in fmol/20 min collection were: Basal 308.8, P. Hypothalamic R: 417.2; Post Ip. R: 568.8 . Infusion of IL-1 produced a very modest nonsignificant 11% increase in hypothalamic NE (in fact 3 of the 5 animals actually showed a decrease). However animals produced a robust increase in hypothalamic NE in all 5 animals (48%). These data suggest that i.p. IL-1 induces a cascade of events which eventually result in an elevation of hypothalamic NE. Further experiments to delineate these mechanisms are underway.
Effect of cytokines on vasopressin and corticosterone releasing factor release from rat hypothalamic and anterior pituitary tissues. A viral paradigm of hypothalamic involvement of nitric oxide mediated signaling. J.Raber*, G.L.Koch and P.E.Bloom, Department of Neuropharmacology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037.

Much attention has focused on the role of selected cytokines as stimulatory factors of the HPA axis and as modulators of hypothalamic vasopressin (VP) and corticotropin releasing factor (CRF) and arginine-vasopressin (AVP) in this response. The amygdala, involved in stress related reactions and the regulation of the HPA axis has been implicated in this role, with in vitro settings used to investigate the influence of selected cytokines on the release mechanisms of CRF and AVP in the hypothalamus and amygdala, and the possible involvement of nitric oxide in this response. CRF and AVP are released in a calcium-dependent manner from both sites, and this release is responsive to acetylcholine, norepinephrine, or high K(+) (50 mM). The norepinephrine-activated AVP release is antagonized by phenolamine, but not by propranolol, while the norepinephrine-induced CRF release is antagonized by both adrenergic antagonists, suggesting an α-adrenergic receptor mediated AVP response and an α- and β-adrenergic receptor mediated CRF release. The acetylcholine-induced release is antagonized by atropine or methanamine, indicating that both muscarinic and nicotinic receptors can mediate the cholinergic responses. The hypothalamus and amygdala. IL-2 stimulated AVP and CRF release in both regions, in a calcium- and dose dependent manner. Nifedipine, which neutralizes nitric oxide (NO) also induced AVP and CRF release. The IL-2- and acetylcholine-induced release is antagonized by N-fumaryl-N-arginine, a potent inhibitor of nitric oxide formation, indicating a role for NO in this release. N-fumaryl-L-arginine does not effect the norepinephrine-induced released release. Finally, IFN-α stimulated CRF and AVP release from both the hypothalamus and amygdala. These data suggest that in addition to the hypothalamus, the amygdala may also play an important role in the bi-directional communication between neuroendocrine and immune systems (Supported by grant MH 47680).

INTERLEUKIN-1 AND ENDOTOXIN INCREASE RELEASE OF NITROGENEPHRINE IN HYPOTHALAMUS. Adrian J. Dunn*, Gregory N. Sperandio and Jan Laviaky, Department of Pharmacology, Louisiana State Univ. Med. Ctr, Shreveport, LA 71130.

Previous studies have indicated that administration of interleukin-1 (IL-1) and endotoxin (lipopolysaccharide, LPS) increased the cerebral metabolism of noradrenaline (NE), with the greatest response in the hypothalamus. To extend these observations, we have now perfused in vivo microdialysis catheters containing NE and dopamine (DA) and investigated the release of DA and NE from the hypothalamus and cortex. Intraperitoneal injection of 5 μg LPS into freely moving rats increased microdialysate concentrations of NE and dopamine (DA) following a lag time. The responses to DA were much smaller than in the hypothalamus, but 100 μg of LPS produced a similar increase in FFM comparable to that of 5 μg in the hypothalamus. These responses were blocked by injection of indomethacin.

IL-1 (1 μg) injected either ip or iv increased microdialysate concentrations of NE in the hypothalamus. Both injection routes elicited similar microdialysate concentrations, increasing slowly to reach a peak after 2-3 hours. This temporal pattern differs from the elevations of plasma ACTH and corticosterone reported for IL-1; iv IL-1 elicited a rapid response with a peak around 20 min, whereas ip injections elicited a slower response with a peak around 2 hours after injection. This suggests that different mechanisms may be involved in the activation of cerebral NE systems in the HPA axis.

TUMOR NECROSIS FACTOR DECREASES THE LEVEL OF VASOPRESSIN BUT NOT OXYTOCIN IN RAT PITUITARY. G. Shvarts, M. D. Fitzsimmons, A. G. Robinson*, Dept of Endocrinology, Univ. of Pittsburgh, PA 15261.

Cytokines derived from the immune system can modulate the activity of the hypothalamic-pituitary-adrenal (HPA) axis and thereby regulate the intensity of the neuroendocrine response. The role of proinflammatory cytokines in hypothalamus and pituitary. IL-1, IL-2, IL-6 in release of ACTH, GH has been well documented, but little is known about the effect of cytokines on arginine-vasopressin (AVP) and oxytocin (OT) secretion. In the rat hypothalamus, AVP but not OT release greater than AVP release. Yet, excess secretion of AVP is a common accompaniment of certain illness, causing excess water retention and hypernatremia. The stimulus for such elevated secretion of AVP has not been clearly defined. We postulated that AVP may be mediated, at least in part, by cytokines. The present study was designed to test whether Tumor Necrosis Factor alpha (TNF), derived from the immune system can stimulate AVP and OT neuronal pathways. TNF (1ug) was injected iv, in conscious, freely moving rats and the levels of pituitary AVP and OT were measured by radioimmunoassay 2 hours after cytokine injection. Pituitary AVP levels were significantly decreased after injection of TNF: baseline values (n=6) were 1211±42 ng/pituitary for control animals and 671±52 ng/pituitary (p<0.012) for animals (n=9) receiving TNF. However, pituitary OT levels were not changed after administration of TNF (p>0.5). These data suggest that the immunosuppressive system (TNF) can specifically regulate AVP depletion in the posterior pituitary. The lack of effect on oxytocin indicates this is not a direct vascular (neurogenic) effect on the pituitary. The 'stress' stimulates OT in the rats, rather is the increased AVP a non-specific response to stress. Rather, there might be a specific neuro-immune interaction between cytokines and vasopressin receptors.
INTERLEUKIN-1α INDUCES CORTICOTROPIN-RELEASING FACTOR SECRETION AND SYNTHESIS FROM NPLC-KC CELLS THROUGH VARIOUS SECOND MESSENGER PATHWAYS

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It is well documented that stress exerts effects on both endocrine and immune functions. The cytokine, interleukin-1 (IL-1), is known to activate the hypothalamic-pituitary-adrenal axis by stimulating release of corticotropin-releasing factor (CRF) and to also play a prominent role in immune function. CRF secretion and synthesis in the NPLC-KC human hepatoma clonal cell line has previously been shown to be increased by IL-1. The purpose of this study was to elucidate which second messenger pathways mediate this effect. NPLC-KC cells were grown in 6 well Costar plates and treated for 24 or 24 hours with 500 pM IL-1β in the presence of the protein kinase (PKC) inhibitor, H-7 (50 μM), the protein kinase A inhibitor, IBMX (5 μM), or the cyclooxygenase inhibitor (Indomethacin; IND, 300 nM). Both cell extracts and secretion media were assayed for CRF-like immunoreactivity in our radioimmunoassay. All 3 inhibitors reduced the IL-1 effect on CRF secretion. This effect was statistically significant (P < 0.05) at 12 hours; although all three inhibitors reduced CRF secretion at 24 hours, only the H-7 effect was statistically significant. Only the PKC inhibitor, H-7 reduced the IL-1-induced increase in CRF synthesis, an effect that was statistically significant at 12 and 24 hours (P < 0.05). Thus, all three signal transduction pathways are implicated in IL-1-induced CRF secretion while only the PKC pathway is implicated in IL-1-induced CRF synthesis. Supported by NIMH MH 42085. The work was also completed while Dr. Kasckow was a Pfizer awardee.


Hyperalgesia resulting from immune activation by bacterial endotoxin is blocked by both IL1α and by section of the hepatic branch of the vagus nerve (Watkins et al., 1994). Thus, some information about immune activation is conveyed to the central nervous system via peripheral nerves presumably sensitive to circulating cytokines. To determine the site of action of the IL1α, we incubated cryostat sections of rat liver and hepatic vagnus with biotinylated IL1α. The IL1α labeled vascular and connective tissue, and areas known to express IL1 receptors. The biotinylated IL1α also labeled a subpopulation of cells in the paraganglia imbedded in the hepatic branch of the vagus and in the connective tissue of the liver hilus. These cells appear to be chemoreceptive, and are innervated by the vagus nerve (Prechtl & Powley, 1985). These preliminary results suggest that paraganglia are sensitive to circulating cytokines associated with immune activation, and convey this information to the brain via the vagus nerve. Supported by Synergien and NIH NS31569, MH14617.

REPRODUCTIVE REGULATION: CHOMORECEPTIVE MECHANISMS

ATP-SENSITIVE POTASSIUM CHANNELS ARE FUNCTIONAL IN NORMOXIA IN THE RESPIRATORY NETWORK OF ADULT CAT.

K⁺ channels sensitive to intracellular levels of ATP participate in the cellular response induced by oxygen deprivation. These channels are activated when intracellular levels of ATP are reduced or closed when ATP levels are normal. In some central nervous structures they might play a role in the hyperpolarization of cells induced by hypoxia has been described (Mourre et al. 1989; Brain Res. 496, 159). However, a functional role for such channels has not been described in respiratory neurons of adult mammals. Therefore we attempted to identify the presence and functional role of the ATP-sensitive K⁺ channels in the respiratory network of adult cats.

Experiments were performed in anesthetized, paralyzed, vagotomized and artificially ventilated cats. PO₂ and PCO₂ were monitored continuously and kept within physiological ranges to ensure normoxia. Expiratory neurons were recorded intracellularly with glass micro-electrodes, filled with a solution of ATP (5mM) in K-CH₃SO₃ (1.5M) in order to study the effect of ATP injection. A specific agonist (diazoxide, 2-20mM) and 2 specific antagonists (tolbutamide, 2-20mM; glibenclamide, 5mM) were applied topically over the respiratory neuronal pool.

Fourteen cells tested for intracellular injection of ATP showed a membrane depolarization of 4-12mV. Application of the channels with diazoxide produced a hyperpolarization of 3.1±0.05mV in 12 of the 16 tested. These cells were in input resistance not different from adult normoxic behavior of 4-6mV. However, when ATX (K⁺ channel activator) was blocked by tolbutamide or glibenclamide evoked a depolarization of 3.4±0.2mV in 10 of 14 cells tested with an increase in input resistance of 29.3±8.6mV (n=8).

We conclude that ATP-activated K⁺ channels are present in respiratory neurons of adult cats. 2) some of the channels are activated in normoxia and 3) opening of the remaining channels might contribute to the respiratory response during hypoxia.

This work was supported by DFG (RI 27876-11 and RI 27876-11-1).
393.3

DOES HYPOXIA-INDUCED INHIBITION OF GLOMUS CELL OUTWARD CURRENT CAUSE INCREASED CHEMORECEPTOR NERVE ACTIVITY
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One major theory of chemotransduction postulates that hypoxia inhibits a glomus cell potassium current, resulting in depolarization, calcium influx, secretion, and, thus, increased nerve activity. To test this theory, we used an in vitro, superfused glomus cell membrane perfusion system. Experiments were conducted using rat carotid body glomus cells in vitro, superfused with HEPES-buffered saline at 30°C. The perfused glomus cell was identified based on their I/V profile: small inward currents and large, voltage-dependent outward current (760±88 pA @40mV, n=10). Repeated measurements of baseline currents and nerve activity demonstrated that hypoxia (30-60s duration, PO2=0 Torr, at nadir) caused a large increase in nerve activity (15.4±4.3× baseline, p<0.01), but no significant change in holding current (p>0.05). Inward outward currents were significantly decreased during hypoxia (p<0.01), but the magnitude was small (36±17 pA) and did not recover following reoxygenation despite the return of outward sodium activity. Administration of the K+ channel blocker TEA (20mM) caused a large reduction in outward current to 54% of control (p<0.02), but caused no significant change in inward sodium activity (p>0.05). These results demonstrate an independence between changes in glomus cell outward currents and inward nerve activity, and, thus, suggest that hypoxia–transduction is not primarily mediated by modulation of glomus cell K+ conductance.

393.4

IMMUNOHISTOLOGICAL DIFFERENTIATION OF CULTURED CAT CAROTID BODY CELLS. M. Shirahata*, B. Safdolof and R.S. Fitzgerald, Div. of Physiology, EHS, The Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Adult cats have been widely used to study the respiratory and cardiovascular responses to hypoxia as well as the increase in neural activity from the carotid body (CB) responsible for these reflex responses. To understand mechanisms of hypoxic chemotransduction in the CB the availability of functional chemoreceptor cells from adult cats is extremely important. Recently we have developed methods for culturing CB cells which respond to hypoxia for up to one month in culture. However, a critical problem with using cats in cellular studies is identification of type I cells, because (1) the ratio of type I to type II cells is only about 2:1, and (2) both types of cells in culture are similar in size and shape. The purpose of this study was to find surface markers for identification of live type I cells. Three types of cells were clearly distinguished with three antibodies against tyrosine hydroxylase (TH), glial fibrillary acid protein (GFAP) and galactocerebroside (Gal-C). Type I cells were positive only for TH. Type II cells were positive for GFAP, but not for Gal-C or TH, suggesting that type II cells are similar to astrocytes. A third type of cell expressed only Gal-C. Both type I and type II cells were stained with tetraneurin toxin fragment. Since astrocytes in the central nervous system expressed immunocompetent cell markers such as CD21 and MHC-class II, we tested these markers as well. Neither type I nor II cells expressed CD21, but type II cells expressed MHC-class II molecules. These results suggest that live type I cells in culture can be identified by immunohistological methods. Supported by HL 47044 and HL 50712.

393.5

FITICTVE VENTILATORY RESPONSE TO PH CHANGE IN AN IN VITRO BRAINSTEM OF RANA CATESBEIANA. W.G. Filmer1, L. Kuhl1, A.T. Pack1. Center for Sleep and Respiratory Neurobiology and Departments of Anesthesiology1, Animal Biology2 and Medicine3, Univ. of Pennsylvania, Philadelphia, PA 19104

We have developed an in vitro brainstem preparation from the bullfrog, Rana catesbeiana, for investigation of changes in respiratory pHCO2 sensitivity and transmission of related signals within the CNS. In this study we assessed whether extracellular pH changes within a physiologically relevant range in an appropriately placed unit activity of the Vth cranial nerve, regarded as an index of the fictive respiratory motor outflow. Trials consisted of alternating two CO2 bicarbonate buffered mock CSF solutions with pH of 8.10 and 7.60 ±0.05 pH units, respectively, made three times for each of the six preparations studied. For fictive lung ventilation cycles, the periods and area under the curves were measured. All of the preparations demonstrated an appropriate fictive ventilatory response, defined as an increase in the product of instantaneous respiratory rate and burst area, to the pH reduction. Changes in either the average burst area or the period alone were not consistent. Small, high frequency "buccal" oscillations, when present, demonstrated a decrease in average amplitude with pH reduction, but no change in period. Thus an in vitro brainstem preparation of R. catesbeiana has pH sensitivity and offers an attractive model for elucidation of the basic neuronal mechanisms of central chemoregulatory control of ventilation. (Supported by NIH Anesthesiology Research Training Grant: GM 070612)

393.6

NITRIC OXIDE MODULATES CO2 CHEMORECEPTION IN THE FLMONATE SNAIL. J.S. Feldman, J.C. Lorrimer and P.F. McCarthy. Dept. of Physiology, Dartmouth Medical School, Lebanon, NH 03756. Winkler et al. (Soc. Neurosci. 1976;11:172, 1973) have implicated the interneuronal messenger NO in the feeding and respiratory programs of the aquatic mollusk, L. stagnalis. We studied the role of nitric oxide (NO) in CO2 chemoreception in the terrestrial, pulmonate snail H. aspersa. Using an isolated brainstem-preparation, we compared the effects of the reversible NO synthase inhibitors, L-N-nitroarginine methyl ester (L-NAMe) and NO donors, sodium nitroprusside (SNP), L-arginine (L-arg) and hydroxylamine (HOX), on pre- and postjunctional function during normocapnia and hypercapnia (6% CO2). Previously, we have shown that focal exposure of a discrete area between the visceral and right parietal ganglia to 6% CO2 causes large increases in the diameter of the pneumostome (Pn), the breathing orifice in these animals. The addition of L-NAMe (10 mM) to brain perfusate decreased Pn area during normocapnia and focal hypercapnic stimulation compared to control saline, but did not affect CO2 responsiveness per se. The decreased Pn area resulting from L-NAMe treatment could be reversed by adding L-arg (10 mM). The inactive enantiomer D-NAMe (10mM) had little effect on Pn area during normocapnia and hypercapnia. The NO donors, SNP (400 μM) and HX (1 mM) added to the brain perfusate increased Pn area during normocapnia and focal hypercapnic stimulation compared to control saline, but also had no effect on the responsiveness to CO2. We conclude that NO modulates respiratory activity in the snail, but does not appear to be involved in the CO2 chemotransduction process. (Supported by grants HL 40938, HL 17027 and HL 07449.)
394.1

EFFECT OF HYPOXIA ON RESPIRATORY NEURONS IN THE VENTROROLATERAL PONS OF ADULT RATS. S.K. Cole* and T. F. Dick. Division of Pulmonary and Critical Care Medicine, Dept. of Medicine, Case Western Reserve University, Cleveland, OH 44106-4389 USA.

Bilateral chemical lesioning of the ventrolateral pons in the A5 region abolishes the post-hypoxic ventilatory depression (Coles and Dick, ATS Abstract, 1994). Data from subsequent experiments using extracellular recording techniques indicate that neurons in the A5 region display highly-modulated respiratory activity (Dick et al., Neurosci., 1995). Hypothesis: that if in behaving respiratory neurons in the ventrolateral pons mediate post-hypoxic ventilatory depression, these cells would be excited by hypoxia. The purpose of the present study was to characterize discharge patterns of these neurons before, during, and after hypoxia to acute periods of hypoxia.

Adult, male Synagoge-Dawley rats (n=7) were anesthetized with Equithesin, vagotomized, paralyzed, and ventilated with 100% O2. Glass micropipettes (B=25 MΩ) filled with a mixture of L-glutamate and 2% Fast Green were used for recording pontine neurone activity extracellularly and for marking recording sites. L-Glutamate was iontophoresed to distinguish cell bodies from axons of passage. Respiratory activity before, during, and after hypoxia (8% O2) was recorded from neurons located in the dorsolateral (n=3); lateral (n=1), and ventrolateral (n=10) pons.

Discharge frequency increased clearly in three expiratory phases during and following hypoxia. Surprisingly, activity was remodelled, i.e., the on-set and offset of neuronal discharge shift (n=5), but also cells (n=5) became active in additional phases of the cycle. Changes in activity were evident in all cells; in 13 cells discharge frequency and activity remained stable.

These data suggest that post-hypoxic ventilatory depression is mediated potentially through respiratory neurons in the ventrolateral pons, and that the process may include "remodelling" neural activity in response to hypoxia.

Supported by AHA Fellowship (SKC) and NIH HL 42400 (TED).

394.2

PHRENIC AND HYPOPHALIC NERVE RESPONSES TO SUSTAINED HYPOXIA IN DECATERBRATE CATS. M. A. Hunter, M. J. Wasielewski, R. J. Jover, C. T. Leiter, and D. Burton, Jr. Dept of Physiology, Dartmouth Medical School, Lebanon, NH 03756.

A biphasic ventilatory response is observed in adults and newborns of many species during sustained hypoxia; an initial increase in ventilation is followed by a "roll off" of the response to levels greater than or equal to those of the initial peak than the peak response. While the mechanism responsible for hypoxic rolloff is uncertain, previous reports suggest that it is both centrally modulated and dependent on peripheral chemoreceptor stimulation. Furthermore, the depressive effects of hypoxia differs between muscles of the upper airways and the diaphragm in pigs (Martin et al., JAP 0623-672-677). In this study, we questioned whether hypoxic rolloff, in the absence of changes in baroreceptor input and end tidal CO2, was demonstrable in the adult decorticate cat, and if so 2) whether hypoxic depression is expressed equally in hypoglossal and phrenic nerves. We measured hypoglossal and phrenic nerve activity in 16 paralyzed vagotomized mechanically-ventilated decorticate cats while arterial blood pressure and end tidal CO2 were held constant.

Following ventilation with 100% O2, inspired O2 was lowered to 12, 14 or 16% for 10-20 minutes. Nerve activity increased to a maximum of 17% above control before returning to the pre-hypoxic level after 7 minutes of hypoxia. Phasic hyperglossal nerve activity increased to a maximum of 40% above control and remained 25% above control after 7 minutes of hypoxia. There was no correlation between peak nerve activity and the decay constant for rolloff in either nerve. We conclude that hypoxic rolloff can be elicited in adults in the absence of changes in the pons. Moreover, the hypoglossal nerve is more resistant to the depressive effects of hypoxia compared to the phrenic nerve in cats. (Supported by grants HL-01998, HL-19827 and HL-03540).

394.3


Progressive hypoxemia in anesthetized, peripherally chemodenervated cat results in a marked decrease in phrenic nerve activity culminating in phrenic silence and, eventually, gasping. These changes reverse during reoxygenation. To determine if changes in the PN power spectrum correspond to changes in temporal patterning, we examined autoregressive (AR) spectra of 100 msec bins of PN in 4 anesthetized, glomeronotomized, vagotomized cats during isocapnic hypoxia and reoxygenation. The AR spectra had peaks in the 30-60 and 60-120 Hz ranges. Hypoxia resulted in power loss at all frequencies and a shift of the 30-60 Hz peak frequencies <30 Hz. Gasping resulted in increased power in all peaks but peaks were seen only in the 0-30 and 60-120 Hz ranges. During isocapnic recovery, the PN reverted from gasping to eupnea. Phrenic amplitude, initially 200-400% of pre-hypoxic values, returned to control values after 30 min. Power was initially increased in all peaks and returned to control levels over the same period time. The AR spectra of the PN during reoxygenation was initially gasp-like with significant power only in the 0-30 and 60-120 Hz ranges. During return to eupnea, low frequency power shifted from 0-30 to 30-60 Hz ranges. The AR spectra during intermediate recovery had characteristics of both eupnea and gasping, i.e., significant power in both the 0-30 and 30-60 Hz ranges. These results suggest that hypoxia results in a reversible reconfiguration of the central respiratory pattern generator.

(Supported: HL-44678, HL-16022, AHA/NJ).

394.4


We examined activity changes on the rostral ventral medullary surface (VMS) of 5 non-anesthetized, unrestrained goats during poikilocapnic hypoxia (10% O2 in N2) using optical imaging procedures. Under sterile surgery, a coherent fiber probe with a charged couple device was placed over a rostral VMS region which elicited ventilatory and blood pressure depression during local cooling. Tracheostomy and vascular cannulation were performed for assessment of cardiorespiratory measures. Onset of 10% hypoxia was associated with a substantial increase in activation on the VMS, which was sustained for a period of time, and then gradually declined to a lower, stable level of activity as hypoxia was maintained. The temporal trends in neural activity observed are suggestive of the reported ventilatory "roll-off" phenomenon commonly associated with sustained hypoxic challenge.

(Supported by HL-22418, NIDR DE-07212, USPHS-23579 and Parker F. Francis Foundation.)

394.5


Carotid body afferents mediate components of the response to mild hypoxia within the intermediate area of the ventral medullary surface (VMS). Changes in IVMS activity following transient peripheral chemoreceptor stimulation are unclear. IVMS neural activity in 6 spontaneously breathing, pentobarbital-anesthetized cats was measured as changes in 660 nm reflectance from a surgically-exposed IVMS, using a 3.2 mm coherent optical probe attached to a charge-coupled device. Two tidal breaths of 100% O2 or O2 were randomly administered before, and after carotid sinus denervation (CSD). Activity significantly increased (10.1±2.4 %) occurring within 3.1±0.7 sec following O2 onset while decreases in activity (4.8±1.8%) was observed with CSD significantly prolonged the latency of the N response to 7.4±1.1 sec (p<0.01), and increased the magnitude of the response (14.5±2.1%, p<0.01).

Similarly, CSD enhanced the effect induced by O2 (7.2±1.3%, p<0.05) as well as prolonging the latency. We conclude that CSD abolishes the early IVMS responses, although desensitization allows for development of a later IVMS activity response, possibly of central origin. (Supported by HL-22418 & Parker F. Francis Foundation.)

394.6


Previously, we have shown that the inspiratory volume-timing relationship during inspiratory resistive loading is linear, and that increasing levels of hypercapnia significantly lengthen the slope of this relationship. The specific aims of this study were to describe the expiratory volume-timing relationship during expiratory resistive loading and to determine whether this relationship is modulated by elevated CO2 levels. Six chloralose anesthetized cats were presented with three graded levels of expiratory resistive loads (13-540 cm H2O/sec) and tracheal occlusion at three levels of inspired CO2 (room air, 7% CO2, and 9% CO2). The relationship between the control (Vc) and expiratory duration (Tc) can be described by the following equation:

\[ V_c = a + b\cdot T_c + c\cdot T_c^2 \]

The influence of different CO2 levels on this relationship is shown in the following table:

<table>
<thead>
<tr>
<th>CO2</th>
<th>a</th>
<th>b</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room air</td>
<td>-254.63</td>
<td>3.35</td>
<td>-0.0084</td>
</tr>
<tr>
<td>7% CO2</td>
<td>-160.89</td>
<td>1.92</td>
<td>-0.0030</td>
</tr>
<tr>
<td>9% CO2</td>
<td>-167.02</td>
<td>2.23</td>
<td>-0.0034</td>
</tr>
</tbody>
</table>

Following vagotomy, Tc was no longer modulated in response to expiratory resistive loading. The relationship between Tc and expiratory resistive loading can be modulated by hypercapnia and that the modulation of Tc in response to both mechanical and chemical loading is dependent upon intact vagal. (Supported by NIH HD-40369).
ACETAZOLAMIDE INJECTED INTO THE MEDULLARY RAPHE PERNICIOUS NERVE ACTIVITY. D.G. Bernard, A. Li and E.E. Nattie. Dept. of Physiology, Dartmouth Medical School, Lebanon, NH 03756

The medullary raphé was examined for chemoreceptor responses (increased pnicus nerve amplitude (PNA)) to localized tissue acidosis produced by microinjection of acetaclizamide (AZ). Adult male Sprague-Dawley rats were anesthetized, vagotomized, paralyzed, and artificially ventilated. The ventral medullary brainstem was exposed and 1nl injections of mock cerebrospinal fluid (mCSF), AZ (5x10^-5M) or an inactive AZ-analogue (5x10^-5M) were made in the raphé while recording integrated PNA, femoral arterial blood pressure and end-tidal CO2. The injection locations were demonstrated by post-mortem anatomical evaluation. PNA was expressed as percent maximum defined by 9% CO2 stimulation. The mean change in PNA evaluated at 10, 20 and 30 min following 27 AZ injections was greater (p < 0.001; ANOVA) than after 16 control injections. Of the 27 AZ injections, 11 had responses greater than any control response. It appears that the traditional ventral medullary chemosensitive regions may not be the exclusive sites for sensing decreased pH by increasing PNA. Other sites, namely the locus coeruleus and the nucleus tractus solitarii have recently been described and the medullary raphé may be another such chemoreceptor site. (Supported by HL 28066 & HL 26991)

SOMATIC AND VISCERAL AFFERENTS: VISCERAL AFFERENTS

PEROXIDE-SENSITIVE MESENTERIC AFFERENTS: AN IN VITRO STUDY. D.W. Addison, J.W. Lenox and L. Koppel. Scripps Institution of Oceangraphy, UCSD 92093, the Departments of Medicine and Anatomy and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

Receptor fields of single-unit afferents in C-fibers (conduction velocity < 2.5 m/sec) were carefully delimited and characterized on the basis of impulse activity evoked by local mechanical and heat stimuli in an in vitro rat splanchic nerve-sensory preparation. Units were then tested for responsiveness to 1-3% hydrogen peroxide (H2O2) applied to their receptive field. H2O2-responsive units displayed a characteristic discharge pattern comprising windup in discharge rate, decay back to background levels, followed by bursting discharge lasting several minutes. This two-phase response and the latencies involved suggested possible participation of a second cell type. Some units initially unresponsive to H2O2 exhibited characteristic discharge pattern upon subsequent H2O2 stimulation, implying that units responding to initial applications of peroxide may previously have been exposed to sensitizing factors. H2O2 stimulation was observed to unit discharge in response to subsequent mechanical stimulation. Since a number of immunoneurochemical events (neutrophils, mast cells, macrophages) generate reactive oxygen species as a result of activation, the observed sensitivity to peroxide may mediate an interaction between the sensory nerve terminal and activated immunoneutrophils.

Supported by NIH grants NS-5665 and NS-28433.


Injection of the rat cervical vagus nerve with WGA-HRP results in labeled axons and terminals at the light and EM level not only in the brainstem but also in the upper cervical spinal cord. The projection to the cervical cord consisted of a small compact bundle of nonmyelinated axons which was located in the reticular zone immediately lateral to the narrow neck of lamina V and a second, less distinctly organized, set of axons at the medial border of the dorsal horn between lamina I and the dorsal columns. Axons and terminal fields extended from each of these small bundles into lateral lamina V-VII and medial lamina I, respectively. The lamina I terminal fields were compact, lying as small "islands" in the gray matter. All axons identified at the EM level were nonmyelinated. This correlates with calculations of the diameters of vagal spinal afferents suggested by the physiological studies of the cervical respiratory neurons and dorsal horn neurons shown to be affected by vagal stimulation (Dawkins et al., 92; Fu et al., 92). Both simple and glomerular terminals were labeled, with clear vesicles and few dense core vesicles. These results extend the previous evidence (Panneton, 91; McNeill et al., 91; Robertson et al., 92; Kalia and Sullivan, '82) for the presence of afferents from branches of the vagus to the high cervical cord. (NIH grant 3335)


The distribution of calreticulin (CR), a calcium binding protein, was compared with several neuropeptides in fixed frozen cross-sections of rat intestine (30 μm) as well as dorsal root ganglia (DRG) using double-labeling immunochemistry. CR was found to coexist with calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), and substance P (SP) but not with calbindin-D28K and galactin in the fibers overlying the lumen propria of the rat intestinal villi. An acetylcholinesterase (AChE) histochemical stain revealed that the majority of CR cells in the myenteric ganglia were cholinergic and about half of the submucosal CR cells contained AChE but very few fibers in the villi contained both substances. In situ hybridization studies confirmed the presence of CR mRNA in the DRG and enteric ganglia and a ribonuclease protection assay verified the presence of CR messages in the intestine. A small proportion of the CGRP immunoreactive cells in the DRG were also immunoreactive for CR and prior studies have revealed that CGRP coexists with SP and VIP in the DRG. These findings combined with reports that the primary source of CGRP in the rat intestine is the DRG (Sterling and Anderson, Somatosensory and Motor Research, 9-45-59, 1992), suggest the source of the quadruple colocalization is the DRG. While the function of CR within these nerves is unknown, the three potent vasodilatory neuropeptides may influence the uptake of metabolized food products within the vasculature of the villi.
395.5 EFFECTS OF ANTIOXIDANTS ON CISPLATIN-INDUCED EMESIS AND RELEASE OF SEROTONIN IN SUNCUS MUTRINUS

Y. Chen and H. Sato
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Nausea and emesis are frequent side effects during cancer chemotherapy. Antitumor drugs, such as cisplatin, are considered to release serotonin from the enterochromaffin cells that innervate serotonergic 5-HT3 receptors located on the vagus afferents. However, little is known how cisplatin releases serotonin from the enterochromaffin cells. We have proposed the involvement of free radicals in the process (Y. Torii et al. Br. J. Pharmacol. 48, 131, 1955). To assess the role of neurotoxic free radicals and various scavenging drugs on cisplatin-induced emesis and the release of serotonin from the intestines were studied in Suncus murinus, a house musk shrew. Cisplatin (20 mg/kg) was injected intraperitoneally, and the antidepressant was injected subcutaneously either one or 24 hours before the cisplatin. Butylated hydroxyanisole, propyl gallate, ascorbic acid and acetyl-cysteine attenuated the number of vomiting animals and episodes per animal. Acetyl-cysteine also decreased cisplatin-induced release of serotonin in vitro. These results further suggest that free radical-mediated oxidation is involved in cisplatin-induced release of serotonin.

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Previous work showed that axotomy-induced deafferentation altered the numbers of neuropetide and tyrosine hydroxalase (TH) mRNA-containing and immunoreactive neurons in the nodose ganglion. The present study was designed to selectively evaluate the loss of axonal transport on the numbers of vasoactive intestinal polypeptide (VIP), TH, and calcitonin gene-related peptide (CGRP) mRNA-containing and immunoreactive (ir) neurons of the nodose ganglion. Vincristine (VB, 0.15mg/m) was administered to the cervical vagus nerves and the axonal transport was studied using immunocytochemistry. The results showed that vincristine inhibited axonal transport and immunocytochemistry were used to label nodose ganglion neurons at 1, 3, 7, and 14 days after VB treatment. VB treatment decreased the number of VIP mRNA-containing and VIP-ir neurons, and slightly increased the numbers of CGRP mRNA-containing and CGRP-ir neurons in the nodose ganglion. The average labeling density of VIP mRNA-containing neurons increased following VB treatment. VB treatment led to the appearance of low-labeling density CGRP mRNA-containing neurons which reduced the average labeling density of the CGRP mRNA-containing neurons. Application of VB to the cervical vagus nerve decreased the number of TH mRNA-containing and TH-ir neurons in the nodose ganglion. The efficacy of VB to inhibit axonal transport and the absence of VB-induced neuronal damage were verified. These data suggest the presence of an axonally transported influence regulating neuropetide and neurotransmitter enzyme synthesis in mature pacode-derived visceral sensory neurons of the nodose ganglion. (Supported by NIH grant RO1 NS020991)


Peritonitis induced by i.p. injection of acetic acid is responsible for an inhibition of gastric emptying and intestinal transit in rats (1). Purpose: to map spinal and supraspinal pathways that are activated by peritonitis-induced ileus in rats. Methods: male fasted SD rats were injected i.p. either with vehicle (NaCl 0.9%) or with 0.1ml/kg acetic acid (AA) mixed with systemic capsaicin (125mg/kg subcutaneously) and received acetic acid (AA) or saline (i.p. 0.1 ml/kg) injected rats were perfused with 4% FFA. Frozen sections (30μm) of the brain and spinal cord were processed for Fos immunoreactivity (Fos-TRK). Results: no or few Fox-Ir was observed after i.p. injection of the vehicle. Acetic acid induced Fox-Ir in the spinal cord, nucleus tractus solitarius (NTS) and area postrema, parabrachial nucleus, supraoptic and paraventricular nucleus (PVN) of the hypothalamus. A dramatic decrease of Fox-Ir was observed in capsaicin pretreated rats. Conclusions: peritonitis induces c-fos expression in spinal and selective brain nuclei (NTS, PVN) involved in the control of digestive motility in rats. Such expression is vehiculated through capsaicin-sensitive afferent fibers. (1) Rivière P.H.M et al., Gastroenterology 104: 724-731, 1993. (2) Bonaz B. et al., Brain Res. 600: 353-357, 1993.

395.9 C-FOS EXPRESSION IN SPECIFIC AREAS OF THE CENTRAL NERVOUS SYSTEM INDUCED BY PROXIMAL COLON DISTENSION IN CONSCIOUS RATS. V. Martinez, L. Wang and Y. Tachibana, CURE, VA Med. Ctr., Brain Res. Inst. and Dept. Medicine, UCLA. Los Angeles, CA 90027.

Colonic distension evokes C-fos expression in the central nervous system (CNS) of the anesthetized rat. The aim of this study was to characterize the response of neurons in the CNS to proximal colon distension in conscious rats. A flexible balloon (6 cm) was inserted, through a mediastinal laparotomy, into the proximal colon, remaining anesthetized rats. After mechanical stimulation of the colon was performed for 10 min (30 s on, 30 s off), by infusing the balloon with 10 ml of air in conscious rats. In the control group the colon balloon was inserted but not treated. 60 min later the animals were deeply anesthetized and perfused; cryostat sections from brain and spinal cord (lumbar 3,4 levels) were processed for Fos-IR in the CNS. No difference was observed in the lumbar part. These results show that viscerosensory inputs active selective spinal cord and brain areas involved in the integration of autonomic functions, and provide anatomical substrates for the CNS pathways involved in the regulation of colonic function and pain perception. V. Martinez personal support: Ramón Areces Foundation (Spain).

395.10 WHOLE-CELL PATCH CLAMP RECORDINGS OF CULTURED DORSAL ROOT GANGLION NEURON SOMATA THAT INVERSELY INNERVE THE KIDNEY OF THE ADULT RAT. M.A. Vizcaino and W.C. de Groat, University of Pittsburgh, School of Medicine, Department of Pharmacology, Pittsburgh, PA 15261.

Whole-cell patch clamp recordings with intracellular tracing techniques were used to examine the electrophysiological properties ofafferent neurons innervating the kidneys of the adult rats (A). In the fluorescent method (Fast Blue, FB or Fluoroangio, FG) was injected into each kidney 7-14 days prior to the removal of the T8-L2 dorsal root ganglia (DRG) bilaterally. DRGs were dissociated with enzymatic mechanical trituration and individual FB or FG cells were identified with a fluorescent microscope. Renal afferent cells were cultured for 12 hours to 2 days prior to whole-cell recordings. Renal afferent cells were small (major axis mean 18±2μm, range 11-24μm) and had an average resting membrane potential of -50±1.7 mV. Action potentials of these cells were of two types: 1) intradendric (TTX-sensitive, up to spike frequency of 12.6±1.2 Hz) with an inflection on the repolarization phase, 2) TTX-sensitive, shorter duration (7.4±0.9 mV). 64% of renal afferent neurons exhibited low threshold (40 to 45 mV) TTX-sensitive (1 mM) action potentials and Na+ currents. Cells that exhibited TTX-resistant action potentials had high threshold Na+ currents (20 to 30 mV). Some cells exhibited both TTX-sensitive and TTX-resistant Na+ currents. In contrast to bladder afferents these TTX-resistant Na+ currents were present in small and large cells, respectively, TTX-resistant and sensitive Na+ currents in renal afferents were present in similar size neurons (25.8±3.7 μm vs. 26.5±.24 μm, respectively). These results demonstrate that axonal tracing with fluorescent dyes is useful for identifying specific populations of visceral neurons for patch-clamp studies. The results provide the control data for future studies of changes in renal afferent cells following pathology in the kidney. [Supported by NIH grants DK 37241, DK 42369 and NRSK 1 F32 DK 08916-01].
395.11

MUSCULAR HYPERALGESIA FROM URETERAL CALCULUS IN RATS: RESPONSES OF SPINAL CORD NEURONS TO OBSCURE MUSCLE STIMULATION. M.A. Giambenedet, F.A. Dalvi, R. Valente and L. Vecchio, Institute of Medical Pathophysiology, University of Chieti, Italy. Previous studies have shown that rats implanted with an artificial stone in one ureter develop referred hyperalgesia of the ipsilateral oblique musculature which lasts many days. The present study examined input to the spinal cord dorsal horn from the hyperalgesic muscle in 15 stone-implanted rats and from the corresponding normal muscle in 15 controls, using electrophysiological recordings. Contractions were made from 114 single dorsal horn neurons in calculosis rats and 137 units in controls in the T10-T12 segments. Testing was as follows: gentle brushing of the dorso-caudal body surface, noxious skin pinch, graded pressure over muscle, noxious muscle pinch. In calculation rats, 25.66% of the recorded neurons received input from the oblique musculature, with additional input from the skin in 21% of the cases. Of the neurons with muscular input, whether muscular-cutsaneous or purely muscular, 50% displayed background activity and 17.24% were exclusively activated by noxious stimuli. In normal rats, 13.13% of the neurons received input from the oblique musculature, with additional input from the skin in 17% of the cases. Of the neurons with muscular input, 50% displayed background activity and 11.11% were exclusively driven by noxious stimuli. The statistical analysis showed that in calculation rats, there exist a significantly higher number of cells with muscular input was recorded (p<0.001) and, of these cells, a significantly higher number displayed background activity (p<0.001). The results seem to indicate a condition of central sensitization of dorsal horn neurons in rats with referred muscle hyperalgesia from viscera.

395.12


Vaginocervical stimulation (VS)-induced increases in pupil dilatation (PD) and analgesia [as measured by vocalization threshold (VOCT) to tailshock] are reduced, but not abolished, by combined bilateral pudendal, pelvic, and hypogastic neuroectomy (NX). In the present experiment, rats underwent aspiration transection of the spinal cord at levels L5 or T7 to determine if such procedures would replicate the effects of NX, and to determine which of the residual responses, if any, are affected by vagotomy (VX). A control group received a sham operation. VS significantly increased both PD and VOCT to front paw shock, in the SH, L5, and T7 groups. The increases in PD and VOCT in the SH group were significantly greater than those in either the L5 or T7 groups. The differences in VS-induced increases in PD and VOCT between the L5 and T7 groups were not statistically significant. VX abolished the VS-induced increase in PD in the L5 and T7 groups. The effects of VX on the VS-induced increase in VOCT could not be adequately determined after VX due to low survival rate at the time of assessment. In summary, VS-induced responses of PD and analgesia persist after spinal transection at L5 and T7, and subsequent VX abolishes VS-induced PD. These findings provide evidence that a physiological response (PD) to VS is mediated, in part, by the vagus nerve.

Support: NIH-RO1-HD30156.

395.13

EVIDENCE THAT THE VAGUS NERVE MEDIATES SOME EFFECTS OF VAGINOCERVICAL STIMULATION AFTER GENITAL DEAFFERENTATION IN THE RAT. R. Corvus-Rolim, G. Sananes, R. Bianco, L. Gomiero, C. Bryce, R. Whipple, E.J. Mujica-Martinez, and B.R. Komisaruk. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102 and CNICESTAV INP, Apto, Postal 14-740, Mexico, 07000, D. F. MEXICO. In order to ascertain whether any effects of vaginocervical stimulation (VS) are mediated by the vagus nerve, we first transected all obvious afferent nerves from the reproductive tract and then tested for residual responses to VS. After combined bilateral transection of the pelvis, hypogastic and pudendal nerves (NX), the following responses to VS were greatly reduced or abolished: lordosis to flank-perineum palpation, leg extension, immobilization and blockage of both tail withdrawal to radiant heat and leg withdrawal to foot pinch. However, after these nerve cuts, the following persisted as significant responses to VS: 1) Pupil Dilatation (PD); 2) Subdiaphragmatic vagotony significantly reduced the magnitude of this residual response; 2) Analgesia (measured as an increase in vocalization threshold to tail shock), the subsequent vagotomy abolished the analgesia; 3) Heart rate (HR); the subsequent vagotomy produced no significant effect on the HR increase to VS. The present findings provide evidence that the vagus nerve conveys vaginocervical afferent activity that produces pupil dilatation and analgesia. Support: NIH-RO1-TW06394-01A1; 3S06-GM08223-10, Bush Foundation.

395.14

COMPLETE SPINAL CORD INJURY DOES NOT BLOCK PERCEPTUAL RESPONSES TO VAGINAL OR CERVICAL SELF-STIMULATION IN WOMEN. B.R. Komisaruk and B. Whipple, IAB and College of Nursing, Rutgers - The State Univ. NJ, Newark, NJ 07102

In 8 women diagnosed as having complete spinal cord injury (SCI) [4 women @ T6-10, 4 women @ T11-12], and a non-injured control group (5 women), vaginal or cervical self-stimulation (VCS) significantly increased group mean pain thresholds in the SCI groups by up to 74% and in the control group by up to 57%. There were no significant differences among groups in magnitude of this effect. Seven of the 8 women with SCI reported that they experienced sexual cramps. The threshold to perceive cervical self-stimulation per se was significantly higher in the 4/8 women with SCI who perceived this stimulus than in the control group. The perceptual responses to VCS in the women with SCI @ T11-12 may utilize hygoplastic afferent nerves that enter the spinal cord as far rostral as T10; however, the perceptual responses to VCS in the T6-10 group were unexpected. Possible afferent pathways include: 1) intraspinal visceral afferent pathways that remain intact after SCI, are not tested for, and are not part of the criteria for defining "complete" SCI (i.e., absence of voluntary movement, cutaneous pain and touch below the level of SCI); 2) a vaginal afferent pathway from uterine cervix and/or adjacent regions that could bypass the spinal cord and enter the medulla oblongata directly. The latter is supported by recent findings in rats.

Support: NIH: RO1 HD30156.

395.15

PUTATIVE C-FIBER SOMATIC AFFERENTS FROM THE HINDLIMB DO NOT CONVEY THERMAL SENSORY INFORMATION TO NUCLEUS TRACTUS SOLITARIUS (NTS) IN RATS. G.M. Tonev and S.W. Miltin. Department of Pharmacology, University of Texas HSC, San Antonio, TX 78284

Activation of hindlimb somatic afferents elicits NTS neuronal discharge, but the sensory modalities mediating this excitation are unknown. Experiments were conducted in anesthetized rats to determine if hindlimb somatic afferent inputs to NTS convey thermal sensory information. The left hindlimb sciatric nerve was electrically stimulated (1 Hz frequency, 0.5 ms duration) and extracellular unit discharge evoked in the right NTS was recorded. Units were located medially to the tractus, near obex, at depths from 450 to 1100 μm. To categorize unit responses as being a-cfiber or c-fiber evoked, compound action potentials were recorded from the sciatic nerve and the averaged response to 30 stimuli compared at different stimulus intensities (n=3). Short latency excitation, presumably a-fiber mediated, was first recorded at an intensity of ~10 μV and became maximal near 100 μV. Longer latency excitation, presumably c-fiber mediated, appeared near 50 μV and became maximal at intensities of ~400 μV. NTS unit discharge was evoked at intensities averaging ±170 ± 69.5 μV (n=8), suggesting activation by c-fibers. Unit activity was further categorized as c-fiber evoked by injecting capsaicin (10 ng) into the hindlimb arterial supply. In 7 of 8 units, discharge significantly increased from 3.1 ± 1.2 Hz to 8.9 ± 1.5 Hz (p<0.05), while 1 unit failed to respond. Units were then tested for responses to thermal stimuli. Over a 10 second period, non-noxious (40 °C) and noxious (47 °C) heat was applied to the hindlimb. In no instance did heating affect NTS neuronal discharge frequency. These data indicate that NTS units activated by hindlimb somatic nerve stimulation appear to receive c-fiber inputs, but these inputs do not transmit thermal sensory information. (supported by HL 36080)

Neurogenic dural inflammation has been proposed as a source of pain during migraine. This inflammation is produced by the release of inflammatory neuropeptides, resulting in ipsilateral dural protein extravasation from post-capillary venules and increased dural blood flow. Most groups using this animal model of migraine have quantified the amount of protein extravasation using exogenous radiolabeled albumin.

We modified the technique to measure the amount of endogenous albumin leaking into the dural extracellular reaction of albumin with the fluorescent dye, Evans Blue. The dye was injected intravenously in zebrafish pigs, prior to stimulation of the trigeminal ganglia. Following unilateral ganglion stimulation, samples of dura overlying both hemispheres were removed, rinsed, and mounted on a microscope slide. The amount of Evans Blue trapped in dural tissue was measured using a Zeiss fluorescence microscope equipped with a spectrophotometer. This computerized system automatically performed readings on 25 different sites on the tissue and calculated the associated statistical values. Values were expressed as the ratio of average fluorescence in ipsilateral vs. contralateral dura for each animal. This method utilized multiple measurements on each sample which resulted in very precise measurements with a small number of animals per point (n = 3). The ability of several compounds to prevent dural extravasation was evaluated. This model was successful in discriminating the clinically effective and ineffective compounds.


Selective blockade of spinal 5-HT,-receptors strongly potentiates withdrawal reflexes in the decerebrated rabbit, a result which implicates these receptors in tonic descending inhibition (R.L. Clarke & A.K. Houghton, J. Physiol. 433, 19(2), 239-256, 1991). In the present study we have investigated the effects of a selective 5-HT,- receptor agonist, (1)-β-hyderoxy-2-(3-n-propylaminomethyl)-8-(OH-DPAT), on withdrawal reflexes in spinalized rabbits.

Twelve animals were decerebrated and spinalized under halothane/NO anesthesia. The left sural nerve was stimulated electrically to excite selectively Aδ axons only or Aδ + Aδ fibres. Reflexes were recorded from the ipsilateral gastrocnemius muscles (GMS) muscle nerve, averaged, and integrated. 8-OH-DPAT was given iv. in doses of 1, 9 and 90μg/kg, followed by the selective 5-HT,-agonist WAY-100,135 (a gift of Wyeth Research (U.K.) Ltd), also given iv. at 1mg/kg. When high stimulus strengths were used, 8-OH-DPAT always increased reflexes at doses up to 10μg/kg, after which the reflex was a median of 21% (10/10) of resting controls. Increasing the dose of 8-OH-DPAT could increase reflexes further (2 rabbits) or decrease responses (3 animals). WAY-100,135 always had the opposite effect to the agonist. In contrast, reflexes evoked by low intensity stimuli were usually (6 of 7 animals) suppressed after 8-OH-DPAT. In these experiments, the median reflex after the 10μg/kg dose was 46% of controls (n=7, range 10-101%). The reflex increased by WAY-100,135 to a median of 166% of the pre-drug values (range 44-431%). Thus, 5-HT,-receptor activation can facilitate or suppress polysynaptic reflexes, presumably through actions at multiple sites in the reflex pathway. AKH is an SENS critic.


Two classes of presumed pain modulating neurons have been identified in the rostral ventromedial medulla (RVM) of the lightly anesthetized rat. "On"-cells display a burst of activity, and "Off"-cells become quiescent prior to a noxious heat-evoked tail flick reflex (TF). A third class of cells, "neutral," show no TF-related changes in firing.

The role of serotonin within the RVM is not well understood. The aim of the present study was to examine the effects of serotoninergic agonists on the activity of on-, off- and neutral cells. Fifty-three out of 59 cells (99%) responded to 5HT. The predominant effect on all cell classes was a facilitation of spontaneous (45%) and glutamate-evoked (55%) activity. An effect blocked by the 5HT2c antagonist ketanserin. 5HT also enhanced the TF-related on-cell burst. Ketanserin by itself had no effect on cell discharge.

The 5HT1a agonist 8-OH- DPAT (2μm) selectively inhibited the IPSPs in the majority of cells tested. Neurons inhibited by 8-OH-DPAT were on many occasions excited by the antagonistic application of 5HT itself. This effect was similar to that of ketanserin.

These results suggest that individual neurons within the RVM can be both excited and inhibited by serotoninergic agonists. Each receptor subtype shows different behavior to different agonist concentrations. These results confirm that serotoninergic neurons are involved in the modulation of pain processing. Further work is required to unravel the mechanisms involved in the neuronal modification of pain processing.


Serotonin (5-HT) strongly potentiates the spontaneous pain response produced by intraplantar injection of inflammatory mediators (Hong & Abbott, Neurosci., in press). The present study explored the receptor subtype(s) mediating this synergistic effect of 5-HT in algogenesis, and the effects of 5-HT antagonists on formalin-induced pain.

5-HT agonists were injected, alone or with non-steroidal (NSA) or prostaglandin (PG) inhibitors, intraplantarly into the plantar surface of the paws of rats. The behavioral response was recorded using the rating scale developed for quantifying formalin-induced pain. 8-OH-DPAT and 2-methyl-5-HT produced only minor analgesia, and did not interact with PG or NSA. The 5-HT agonists DOI, MCP and 2-methyl-5-HT produced little response alone, but induced lasting and licking lasting more than 30 min with moderate to high doses of NSA. The algogenic effect of 2-methyl-5-HT plus PGE2 was dose-dependently blocked by intraplantar pretreatment with the 5HT,-antagonists ketanserin (50-μg/mg) and ketanserin (50-μg/mg) and ranitidine (10μg/mg) or the 5HT,-antagonists ketanserin (50-μg/mg) and ranitidine (10μg/mg) or the 5HT,-antagonists ketanserin (50-μg/mg) and ranitidine (10μg/mg) or the 5HT,-antagonists ketanserin (50-μg/mg) and ranitidine (10μg/mg) or the 5HT,-antagonists ketanserin (50-μg/mg) and ranitidine (10μg/mg). These results suggest that 5-HT-agonists may be effective in the treatment of pain in chronic pain states, as well as in the processing of nociception (Supported by NS14455, NS28064, NS11255).
396.7

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The clinical success of SHT-receptor agonists has indicated a role for a SHT-like receptor in migraine. Two models have been proposed which postulate either a vascular or neural location for the SHT receptor. MDL 100,687A was characterized in vitro and then evaluated in vivo for their ability, following i.v. administration, to alter blood flow in the carotid circulation (closure of arteriovenous anastomoses in cats) or transmitter release (attenuation of plasma extravasation following intrathecal injection of capsaicin and gain pigs). MDL 100,687A was similar in potency and efficacy to sumatriptan in affecting blood flow (ED50 values were 438 and 726 nmol/kg, respectively). In contrast, respective ED50 values vs. plasma extravasation were 2-4 orders of magnitude lower (0.5 and 5.5 nmol/kg). In addition, the minimally effective dose of MDL 100,687A was 1,000-fold lower than that of sumatriptan in the neurogenic model. Thus, in general, the SHT agonists were more potent against the neural vs. the vascular component of migraine and specifically, MDL 100,687A was exceedingly potent (compared to sumatriptan) against its neural components. These two responses might be having different pharmacological mechanisms.

396.9


NGF has been shown to produce hyperalgesia 24 hr after injection in rats. We tested the effect of 0.1 μg of NGF injected intrathecally in mice. The latency of the tail flick test, a hot water bath maintained at 49°C was significantly decreased at 24 and 48 hrs after injection of NGF. In contrast, the abdominal stretch response (writhing) to an intraperitoneal injection of acetic acid was not altered by NGF pretreatment at these times. Parallel studies indicate that SP-1(7), the predominant N-terminal metabolite of SP, was antinociceptive in the abdominal stretch assay at 24 and 48 hrs. However, a dose of 100 nmol of SP-1(7), injected 24 hr before testing, elicited no change in the hot water tail flick response. In spite of its lack of efficacy in the tail-flick assay, when 1 to 100 nmol of SP-1(7) was administered together with 0.1 μg of NGF, this N-terminal fragment of SP inhibited the hyperalgesic effect of NGF in a dose-related fashion. D-SP-(1-7), a D-amino acid substituted isomer of SP-1(7) that antagonizes SP N-terminal binding and activity, reversed the inhibitory effect of SP-1(7) on NGF-induced hyperalgesia. Together these data suggest that N-terminal metabolic fragments that are presumed to accumulate in the spinal cord during pain transmission serve as long-term modulators of pain by antagonizing the action of NGF. (Supported by NIDA 04090)

396.10


The superficial laminae of the dorsal horn of the spinal cord (laminae I and II) are areas rich in the neuropeptide Substance P (SP). These are the areas where the small lightly myelinated and unmyelinated Aδ- and C-fibers terminate. Activation of these fibers elicits an increase in the release of SP in the dorsal horn of the spinal cord. The opioid peptide met-enkephalin, is also found in high concentrations in the dorsal horn and is a selective δ-opioid receptor agonist. The aim of this study was to determine whether δ-opioid receptors are involved in the control of the release of immunoreactive SP in the dorsal horn when noxious mechanical or thermal stimuli are applied to the ipsilateral hindpaw of the rat.

A push-pull cannula was introduced into the dorsal horn at the lambar enlargement in decerebrate/spinalized rats. The spinal cord was perfused with artificial CSF and immunoreactive SP (SPLI) was measured using RIA, before and after the application of a noxious mechanical or thermal stimulus. All drugs were applied to the dorsal horn through the perfusion apparatus.

ME reduced the tonic release of SPLI. It also bloclked the increase in SPLI following either the mechanical or thermal stimuli. The effect of ME was blocked by nalttradine (NDT, a selective δ-opioid receptor antagonist). NTD alone, elicited an increase in the tonic release of SPLI. These results suggest that there is a δ-opioid receptor mediated control of the tonic as well as the evoked release of SPLI.

396.11


RPR 100863 is a potent and selective antagonist of human Nk1 type 1 (Nκ1) receptors. We used whole-cell patch clamp techniques to evaluate the antagonist activity of RPR 100863 on the peak outward (potassium) current recorded from the human angiotoma cell line U 373 MG. 100 nM RPR 100863 inhibited receptor activation by substance P (SP: 10 nM for 5 secs once every 5 min). At least two control responses to SP were recorded prior to a 10 minute continuous application of antagonist. RPR 100863 (10−12–10−7 M) inhibited SP-induced current in a concentration-dependent manner with an IC50 value of 28 nM. In control experiments, the antagonist effect of RPR 100863 was tested on NK1 receptors, in the presence of 2 μM SP, 100 nM U 373 MG. 100 nM RPR 100863 (10−12–10−7 M) inhibited SP-induced currents (10 μM). The antagonist effect was dose dependent and reversible. These results confirm that RPR 100863 is a potent and stereoselective antagonist of human NK1 receptors, and indicate that this may have slower association and dissociation kinetics than previously described Nk1 receptor antagonists.

396.12

THE SUBSTANCE P N-TERMINAL, HEPATEPIDET, INJECTED 24 HRS BEFORE THE FORMATION OF SP AUTOPHANY INHIBITS THE TONIC PHASE. V.M. Coutiño and A.A. Larsson. Graduate Program in Neuroscience, Univ. of Minnesota, St. Paul, MN 55108, U.S.A.

There is much evidence that substance P (SP) is released in the spinal cord in response to a nociceptive stimulus. Since conditions such as hyperalgesia can occur days or months after nociceptive stimulation, we investigated whether SP produces long-term effects. The SP N-terminus and SP N-terminal metabolites, such as SPI(1-7), produce antinociception in the abdominal stretch assay when injected intrathecally (I.t.) 30 min before testing. However, in the formalin assay, in which mice are injected s.c. with 30 μl of 5% formalin into the dorsum of the rear foot and the amount of time spent licking or biting the foot is counted, SPI(1-7) was without effect in the acute phase (0-5 min) or the chronic phase (20-30 min) of the behavioral response when SPI(1-7) was injected i.t. 5 or 30 min before formalin injection. However, when administered i.t. twenty-four hours before testing, SPI(1-7) produced a U-shaped dose-response curve in both phases. The acute phase was potentiated at 125% and 135% of control at doses of 1 and 10 nmol, respectively. In contrast, the tonic phase was inhibited with decreases to 64% (p<0.05) or 51% (p<0.05) at 0.1 nmol. 51% and 59% at 10 nmol. Ten nmol of SPI(2-11), a C-terminal SP metabolite active at neurokinin receptors, was without effect. These data implicate an accumulation of both the long-term and differential modulation of pain, perhaps contributing to the development of hyperalgesia or allagasia chronically, depending on the type of nociception measured. (Supported by NIDA T32 DA07234 and DA04090)
396.13

**EFFECT OF L-TYPE CALCIUM CHANNEL ANTAGONISTS ON THE RESPONSES OF DORSAL HORN NEURONS TO PERIPHERAL CUTANEOUS STIMULI AND TO SUBSTANCE P IN CATS.** V. Radakrishnan and J.L. Illes*. Departments of Physiology and Psychiatry, McGill University, Montreal, Quebec.

Substance P, a mediator of nociceptive transmission at the level of the primary afferent synapse, is capable of elevating intracellular calcium in dorsal horn neurons. Substance P (NK-1) receptor antagonists, such as CP-96,345 and CP-99,994, have been shown to interact with L-type calcium channels, thereby raising doubts about NK-1 receptor selectivity in blocking nociceptive responses. Therefore, in the present study we have tested the effects of L-type calcium channel blockers, verapamil and diltiazem, on the responses of spinal dorsal horn neurons to peripheral cutaneous stimulation and to iontophoretic application of substance P. Extracellular recordings were obtained using multibarrel electrodes from L4-L6 segments of the spinal cord in cats anesthetized with α-chloralose and spinalectomized at the L1 level. Substance P, verapamil and diltiazem were applied to the neurons by iontophoresis. Verapamil and diltiazem depress spontaneous activity by 20-40% in 8/10 neurons tested. The response to hair stimulation was depressed (20-50%) in 7/10 neurons. Responses to noxious mechanical and noxious thermal stimuli were depressed (40-60%) in 6/6 neurons. The response to substance P was also blocked by verapamil and diltiazem in 5/6 neurons. Thus, in contrast to NK-1 receptor antagonists which did not affect the spontaneous firing or the response to hair stimulation at doses that blocked the response to substance P, L-type calcium channel antagonists showed a suppression of all types of responses, irrespective of the intensity. Additionally, it is likely that the antagonism of nociceptive responses by CP-96,345 and CP-99,994 is unrelated to their interaction with calcium channels. (Supported by MRC of Canada and Pfizer Central Research)

396.15

**HYPERALGESIA INDUCED BY SPINALLY ADMINISTERED CELL PERMEABLE ANALOGS OF CYCLIC 3'5'-GUANOSE MONOPHOSPHATE (cGMP)** Ethin Abraham*, Mary G. Garry, Kenneth M. Harkevets and Lin Aanoners†. 1st of Biology, Macalaster College, St. Paul, MN 55105; 2nd, Dept. of Restorative Sciences and Pharmacology, University of Minnesota, Minneapolis, MN.

Recent studies have shown that spinal levels of cyclic 3'-5' guanosine monophosphate (cGMP) are greatly involved in the development of hyperalgesia. The purpose of the present study was to investigate whether direct, spinal application of cell permeable cGMP analogs would result in a hyperalgesic response as measured in the hot-plate assay. Male, NDS Swiss mice (16/group) were injected intrathecally (i.t.) with 8-bromoguanosine 3'-5'-cyclic monophosphate (8-bromo-cGMP, 100 µg) or saline (baseline). If (i.t.) 1) if there was a hyperalgesic effect (a decreased latency to lick the hind-paw) and 2) the time course of the effect. 8-bromo-cGMP produced a significant hyperalgesic response when compared to a saline control from 3 to 30 minutes post-injection. Maximal effects were observed at 10 min., thus, this time was used in subsequent studies. 8-bromo-cGMP and N2,2'-O-dibutyrylguanosine 3'-5'-cyclic monophosphate (db-cGMP) produced dose-dependent hyperalgesia with ED50 of 0.69±1.59 mmol/ml and 0.79±1.11 mmol/ml respectively. The selectivity of this response was determined by observing the response to i.t. injection of other cell impermeable guanosine compounds and CAMP. The other guanosine compounds did not have an effect, while a CAMP significantly increased hot-plate latency. These results suggest that spinal cGMP may be involved in the facilitation of thermal hyperalgesia in mice.

396.16

**ACTIVE PROTEIN KINASE C INCREASES RELEASE OF NEUROPEPTIDES FROM RATTLE SENSORY NEURONS.** L.A. Barber* and M.R. Vasko, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana 46202.

Although activation of protein kinase C (PKC) has been shown to enhance excitability of sensory neurons, it is not known whether this activation alters the release of neuropeptides from sensory neurons. We have recently shown that PKC alters the release of substance P (SP), and calcitonin gene-related peptide (CGRP) from rat sensory neurons in culture. To further define the relationship between PKC and neuropeptide release, we have employed a number of PKC inhibitors to determine the PKC dependency of SP and CGRP release from rat dorsal root ganglia neurons. Sensory neurons were dissociated from fetal rat dorsal root ganglia (E19-17) and grown in culture for 9-12 days. Release experiments were performed using neuronal cultures for 10 minutes in the presence of ionophore A23187 (10µM) and 1mM ATP. The PKC inhibitors were staurosporine, H20118, and G50286. These agents were added at concentrations of 10-4M to the perfusate containing 1mM ATP and 10µM of ionophore. SP and CGRP release were measured by RIA and HPLC, respectively. The results show that PKC and CGRP release are enhanced by ionophore-induced calcium influx. The results suggest that PKC activation is involved in the augmentation of release of SP and CGRP from sensory neurons, and that this mechanism is important in the regulation of neuropeptide release from these neurons.

396.17


Inflammatory mechanical hyperalgesia induced by tissue injury (e.g. exposure to carrageenan) is due to activation/sensitization of sensory nerve terminals by mediators which are also released by mast cells. Tissue damage is also reportedly associated with increased generation of the N-acyl-(2-hydroxyethyl)hexadecanamide, recently proposed to behave as a local autacoid capable of negatively modulating mast cell activation. To test this concept, treated and untreated rats were administered intrathecal (i.t.) saline or a nanomolar dose of this compound. The N-acyl-(2-hydroxyethyl)hexadecanamide, recently proposed to behave as a local autacoid capable of negatively modulating mast cell activation. To test this concept, treated and untreated rats were administered intrathecal (i.t.) saline or a nanomolar dose of this compound. The N-acyl-(2-hydroxyethyl)hexadecanamide, recently proposed to behave as a local autacoid capable of negatively modulating mast cell activation. To test this concept, treated and untreated rats were administered intrathecal (i.t.) saline or a nanomolar dose of this compound. The N-acyl-(2-hydroxyethyl)hexadecanamide, recently proposed to behave as a local autacoid capable of negatively modulating mast cell activation. To test this concept, treated and untreated rats were administered intrathecal (i.t.) saline or a nanomolar dose of this compound. The N-acyl-(2-hydroxyethyl)hexadecanamide, recently proposed to behave as a local autacoid capable of negatively modulating mast cell activation. To test this concept, treated and untreated rats were administered intrathecal (i.t.) saline or a nanomolar dose of this compound. The N-acyl-(2-hydroxyethyl)hexadecanamide, recently proposed to behave as a local autacoid capable of negatively modulating mast cell activation. To test this concept, treated and untreated rats were administered intrathecal (i.t.) saline or a nanomolar dose of this compound. The N-acyl-(2-hydroxyethyl)hexadecanamide, recently proposed to behave as a local autacoid capable of negatively modulating mast cell activation. To test this concept, treated and untreated rats were administered intrathecal (i.t.) saline or a nanomolar dose of this compound. The N-acyl-(2-hydroxyethyl)hexadecanamide, recently proposed to behave as a local autacoid capable of negatively modulating mast cell activation. To test this concept, treated and untreated rats were administered intrathecal (i.t.) saline or a nanomolar dose of this compound. The N-acyl-(2-hydroxyethyl)hexadecanamide, recently proposed to behave as a

Incapable tailshock produces supersensitivity to morphine analgesia (see companion abstract). The addictive nature of supersensitivity is in agreement with previously reported learned helplessness effects. Since several learn and unlearn procedures can be prevented by opiate antagonists or benzodiazepines prior to inescapable shock, these drugs were used to determine if they would also prevent supersensitivity to morphine analgesia.

Rats were injected ip with 5 mg/kg diazepam, 10 mg/kg naloxone, or vehicle. Rats then received either 100 tailshocks (5 x 1 min variable ITI, 1 mA) or an equivalent period of restraint (control). Rats were then used for pain responsivity (tailflick TF) tests in a novel environment 24 hrs later. Rats were first assessed for baseline pain responsivity (3 TF), followed by either 1 mg/kg s.c. morphine or 1 mg/kg s.c. sterile saline. 

Beginning 15 min later, 3 TF intensities were assessed each min for 60 min. Regardless of drug received prior to shock, no analgesia was observed in any group injected with s.c. saline at time of pain testing. Likewise, no analgesia was observed in any morphine-injected restraint control group. In contrast, profound morphine analgesia was observed in vehicle-injected rats exposed to tailshock 24 hrs previously. This supersensitivity to morphine analgesia was prevented in rats which received either naloxone or diazepam at the time of inescapable tailshock.

These data re-interpret supersensitivity to pharmacological morphine analgesia induced by inescapable tailshock. Prevention of these effects by opiate antagonists & benzodiazepines at time of shock is in agreement with other previously reported learned helplessness phenomena. NIH MH351470

397.1 GROWTH FACTOR STIMULATION OF OPSIN IMMUNOREACTIVITY IN CULTURE FROM ROD PHOTORECEPTORS FROM THE RODLESS MICE. J. C. Blank*, E. Barron, S. Y. Schmidt, Y. Courtois and D. Hidu. Doheny Eye Institute, Los Angeles, CA 90033; INSERM, Paris, France.

Retinal cell cultures from postnatal day 1 (P1) control and rd mice were used to assess the effects of various growth factors on neuronal differentiation. In rd retinas, the photoreceptor degenerate during early development due to a deficiency in a specific opsins. Our objective is to develop an in vitro system in order to enhance photoreceptor cell survival and/or differentiation. Retinal suspensions from P1 control and rd mice were grown on coverslips at 5 x 10^5 cells/coverslip under basal conditions or in the presence of bFGF (10 ng/ml), NGF (50 ng/ml), EGF (20 ng/ml) or bFGF and NGF combined. Photoreceptor cell viability in cultures of control and rd mice were evaluated by counting the numbers of opsins-positive cells (opsin+) throughout the entire coverslip after 4 days in culture. The numbers of opsin+ cells were similar in control and rd cultures, and in both there was a two- to threefold increase relative to baseline in cultures with bFGF and NGF. EGF had no effect on photoreceptor numbers. There did not appear to be a synergistic effect when bFGF and NGF were combined. Cultured cells survived for a minimum of 9 days. Double-label studies are in progress with opsin and neuron-specific enolase (NSE) antibodies to determine if bFGF and NGF have a specific effect on photoreceptor cells or if their effects extend to other neuronal cell types in the retina labeled by NSE. The finding that opsin+ photoreceptor cell numbers were increased by growth factors in rd as in control retinal cultures raises the possibility that bFGF and NGF may also help the survival of rd photoreceptors.


Phototransduction in retinal rods involves a signaling cascade that leads to cGMP hydrolysis and the closure of cGMP-gated cation channels open in darkness, producing a membrane hyperpolarization as the light response. For many years there has also been some evidence for the presence of a phosphoinositide pathway in the rod outer segment, though its function and the molecular identities of its components are still unclear. We have studied this problem with immunocytochemistry using antibodies against phosphoinositide-specific phospholipase C (PLC) isoforms and also alpha subunits of the Gq family of G proteins. Among the PLC isoforms we have examined (1), 3 and 1, only PLC4-like immunoreactivity was found in both rod and rod outer segments. At the same time, we have localized Gq11, a member of the Goq family, also in the rod outer segments of the same species. Immunoblotting of total retinal proteins with anti-PLC4 and anti-Gq11 antibodies also gave a single protein band in crude rod retinal and rod outer segments. At the same time, we have localized Gq11, a member of the Gq family, also in the rod outer segments of the same species. Immunoblotting of total retinal proteins with anti-PLC4 and anti-Gq11 antibodies also gave a single protein band in crude rod retinal and rod outer segments. We conclude that PLC4 and Gq11 are likely to be part of the phosphoinositide signaling pathway in the rod outer segment. Biochemical studies are in progress.


We examined previously that the expression of the rhodopsin promoter linked to an attenuated diptheria toxin gene, eliminates rod photoreceptors in the retina of transgenic mice, leaving what appear morphologically to be cone somata in the outer plexiform layer (OPL). In recent studies, we have obtained further evidence that the cells that remain in the OPL of our transgenic mouse retinae are cones, and, therefore, that the direct effect of this genetic ablation is limited to the rods. In one set of experiments, we used reverse transcription PCR to quantify the levels of rod and cone opsin transcripts. In our "rodless" mice, rhodopsin expression is lower at 14 days postnatally than in age-matched controls, and rhodopsin cannot be demonstrated after 21 days. Blue cone opsin expression in the "rodless" retines, while lower than in controls after 35d, can still be detected in "rodless" adults. Similar experiments are now being performed to quantify the levels of rhodopsin opsin remaining after rod ablation. We also used peanut agglutinin to selectively label cone and cone outer segments in our "rodless" mice. The proportion of labelled somata in the OPL increases with age, and only labelled somata remain at 35d. In addition, we crossed our "rodless" mice to transgenic mice in which bipolar and ganglion cells express the 8-gal-gene. The number of bipolar cells in these retinae appears normal. Their location within the inner nuclear layer (INL) appears altered, however. Labelled bipolar cells are no longer found only near the outer margin of the INL, but are distributed throughout this layer. The selective loss of the rod photoreceptors also does not appear to affect the number of retinal ganglion cells. Supported by NIH EY10009 & EY04977.

397.4 DOCSOSAHEXANOIC ACID UPTAKE IN THE CONE-DOMINANT LIZARD RETINA. W.C. Green*, H.F. Baehr3 and N.G. Baehr. LSU Neuroscience Center, LSU Medical Center, New Orleans, LA 70112.

The retina effects the high-affinity uptake of docosahexaenoic acid (DHA). Photoreceptors actively accumulate DHA for synaptic and photoreceptive membranes. Light- and electron microscope-level autoradiography of [3H]DHA has shown that from 63% (primates) to 92% (amphibia) of total retinal label occurs within photoreceptors. In the frog, rod photoreceptors incorporate much more DHA than cone photoreceptors, but [3H]DHA labeling of rods and cones is similar in monkey and rabbit retinas. To determine if some cone photoreceptors are more similar to rods, we used the American chameleon (Anolis carolinensis), which has a cone-dominant retina with a pronounced foveal region consisting of morphologically distinct, long, slender cone cells, and a peripheral region of shorter, wider cones. As controls, we used an all rod retina, from the mediterranean gecko (Hemidactylus turcicus), and the frog (Rana pipiens). Retinas were incubated 4 h in 2 µCi/ml [3H]DHA (SA 23 C/mmol). Half of each retina was prepared for lipid analysis, the other half for autoradiography. Animals of each species gave a good, rod-based pattern. Our data revealed that DHA mole percent content was similar in frog and gecko (32%) but only half in chameleon (18%). von Scante et al. (Jovet. Ophthalmol. Vis. Sci. 32:2558-2566, 1994) recently described rod-cone similarities in the cone-dominant squinted retinas at a molecular level. Based on these differences among the cell types of the animals studied, we suggest that some cone photoreceptors may possess pathways of phospholipid metabolism that are closer to those found in rods. (Supported by NIH NEI EY05121)
397.5

IDEAL OBSERVERS OF NEURAL SIGNALS IN PHOTO-
TRANSMISSION AND THE NOISE CHARACTERISTICS OF A
PHOTOCONDUCTOR. J. D. Immel, J. Stavrianos, and R. L.
Winlow*, Dept. of Biomedical Engineering, Johns Hopkins
University School of Medicine, Baltimore, MD 21205

The rod phototransduction model of Forlì et al. was linearized
about operating points corresponding to various background light
levels. The linearized model was used to predict low frequency
quantal noise in the outer segment current. The just noticeable
difference (JND) in intensity (in units of photoreceptor/s) based
on observation of outer segment current in response to 200 msec
light flashes was determined at different background levels using
ideal observer theory. Performance was compared to that
predicted based on optimal processing of photon counts.

Results show that the linearized Forti model of phototransduction
represents features of low frequency outer segment current noise
measured experimentally, such as: a) the magnitude of the noise is a
non-monotonic function of the background light level, with the
maximum occurring at a dim illumination level; and b) trapping
BAPTA (a calcium chelator) in the rod increases noise level.

Ideal observer analyses indicate that intensity JNDS based on
observation of outer segment current in response to light flashes are
5-10 times higher than JNDS based on photon counts. The
phototransduction process therefore results in an information loss
at the earliest stages of neural processing within the retina.

397.7

RP-CAMPS: A COMPETITIVE ANTAGONIST OF CYCLIC NUCLEOTIDE-
GATED CHANNELS. Richard H. Kramer* and Gareth T. O. "Dept. of
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33101, and Biological Psychology, SUNY, New Paltz, NY, 10033

Cyclic nucleotide-gated (CNG) channels generate the primary electrical
signal in photoreceptors and olfactory neurons during sensory responses.
Cyclic nucleotides bind to a site on CNG channels that is homologous to the
cyclic nucleotide binding domain of CAMP- and CAMP-dependent protein
kinases. Therefore we investigated the effect on CNG channels of thioate
derivatives of CAMP and CCGMP (RP-cAMPS and RP-cGMPS; competitive
antagonists of CAMP and CAMP antagonists, respectively). In CNG
channels from catfish olfactory neurons (OLF channels), RP-cAMPS is a partial
agonist. Alone it only weakly activates OLF channels, but strongly
agonizes activation by co-applied CAMP or CCGMP. The inhibition is
competitive, with a large effect at low CAMP levels and no effect at saturating
CAMP. Single channel analysis shows that RP-cAMPS reduces open probability
without affecting single channel conductance. In contrast, Sp-cAMPS, the
diester of RP-cAMPS, is a full agonist of OLF channels. One might
expect RP-cAMPS to antagonize activation of rod photoreceptor CNG
channels (RET channels), since these channels are selectively activated by
cAMP. However, RP-cAMPS is an agonist, activating up to 80% of the current
elicited by saturating CAMP. Surprisingly, RP-cAMPS is a pure antagonist of
RET channels, exhibiting a K_i of 200-500 mM. RP-CP-cAMPS is an even
higher affinity antagonist that is also membrane-permeant. Extracellular
application of this analog may be useful for investigating the functional role
of CNG channels in these and other cell types. Supported: NIH grant NS36695.

397.9

MULTIPLE CALCIUM CHANNEL SUBTYPES IN TIGER
SALAMANDER CONE PHOTORECEPTORS. M.E. Wilkinson* and S.
Barney, Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Numerous studies confirm that photoreceptor Ca channels, activating
near -40 mV and showing little if any inactivation, are of the high-voltage-
activated, low-threshold type. In this study, two possibilities for the
presence of cone photoreceptors bathed in 5 mM BaCl_2 revealed HVA Ca channel
subtypes. The majority of Ca channel current was L-type as it was blocked in
a voltage-dependent manner by 8-400 μM nifedipine (0.1 - 100 μM),
but a component of Ca channel current was resistant to even the highest
dose of nifedipine. Kinetic properties of the current
component activated in 10 μM nifedipine seemed to support the idea that
activation occurred faster and more inactivation was seen. Most of this
component was reversibly blocked by w-conotoxin GVIA (1 μM),
indicating that these Ca channels are of the N-type. The voltage range of
activation was indistinguishable for L- and N-type. Bag K 8464 enhanced the
Ca channel current markedly and increased the time constant of tail
current deactivation from 10 μM to 100 μM. Application of w-conotoxin GVIA
(1 μM) to Bag K treated cells blocked a smaller proportion of current during
the depolarizing test step and had no effect on the slowed tail
current, suggesting that the agonist was not blocking L-channels. The
P-channel blocker w-agatoxin IVA (200 nM) had no statistically significant
blocking effect. A small current component that could be blocked by
CoCl_2, persisted in the presence of all three channel type blockers.

Supported by the Medical Research Council and the Alberta Heritage
Foundation for Medical Research.

397.6

D_A Dopamine receptors, negatively coupled to adenylate
cyclase. Block the hyperpolarization activated current
(h) in mammalian rod photoreceptors. B. Lengyel*, G.G.
E Diplomatura Fisica, Pisa, Ist. Neurofisiologia, CNR, Pisa, ITALY.

The voltage response of retinal rods reflects both the suppression of the
whole-cell current entering at the outer segment and the activation of voltage dependent
conductances of the inner segment. We have measured the voltage
dependent currents and the membrane potential shift of retinal rods by using the
whole-cell voltage clamp technique. We found that the main component is an
inward current (i_h) activated by stepping the membrane potential to voltages
between -70 and -140 mV of the resting background. In an average of 200 - 500 M
BAPTA rods the half-activation voltage from -90 to -70 mV. Dopamin 1-10 μM
reversibly shifts the half-activation voltage of i_h from -90 to -105 mV, an effect
opposite to i_h application. The results suggest that dopamine receptors,
located on mammalian rod inner segments, inhibits the adenylyl cyclase.
The properties of dopamine receptors were further investigated on membrane
homogenates obtained from bovine rods, after isolation by mechanical
dissociation and purification on Ficoll gradients. By using the [H]-spiperone
binding assay we found the specific binding to be saturable and of high affinity
with a K_d of 0.05 nM. The K_i obtained from antagonist displacement curves are
consistent with the known pharmacology of D_A-like receptors, moreover the
K_i for clozapine (40 μM) is typical for D_A receptors. Biphasic displacement
curves for agonists suggest that dopamine receptors are coupled to an
endogenous G-protein (probably GTP.) The receptor from an
high affinity state for agonists to a low affinity one. We conclude that dopamine
affects the membrane properties of mammalian rods by activating D_A
receptors which are negatively coupled to adenylate cyclase.

Supported by the EC (SC1-0224-C) and MIP 40% to L.C.

397.8

ROD PHOTORECEPTOR M-CURRENT (i_M) IS STABILIZED BY CALCIUM-DEPENDENT PROTEIN KINASE.
D.E. Karpenko and S. Barnes*, Neuroscience Research Group,
University of Calgary, Calgary, Alberta, Canada T2N 4N1

When recorded with conventional ruptured-patch whole cell recording
in bright light, kinetic parameters of the non-inactivating voltage-sensitive
rod M-current (i_M) change gradually ("run down") in the following
manner: the maximum current amplitude and time constant of current
activation decrease, while membrane potential shift of the current
increases. In contrast, i_M was stable during ruptured-patch recording in complete
darkness or when using the perforated patch technique. However, the
stability of i_M could be maintained in ruptured-patch recordings by bright
light when the intracellular calcium concentration was increased by using either
calcium-BAPA buffer ([Ca]_o = 1 μM), or caffeine (1 mM). IP, a
second messenger that elevates calcium from intracellular stores stabilized
i_M when included in the pipette solution at 10 μM but not at 1 μM.
Including the phorbol ester PMA in the pipette solution (20-100 nM) also
prevented rundown of i_M but in only 50% of cells. The protein kinase C
inhibitor, H-7, added to the pipette solution (1 μM) prevented the receptor from an
Ca (Ca-BAPA buffer) restored i_M rundown. 1,2-dioctanoyl-sn-glycerol
(50 μM) KPI/AEG (10 μM) and CAMP (200 μM), ATThS
or GTPjS (up to 1 μM) and intracellular calcium
levels to stabilize i_M. None of the drugs caused any effect on i_M when applied
extracellularly. We conclude that a calcium-dependent substance
inhibited by H-7, probably protein kinase C, is present in photoreceptors
and is necessary for i_M stability.

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397.10

Extracellular pH and CI Concentration modulation independently
Ca^2+ Currents in Tiger-Salmanders' Photoreceptors. B. Nitzan* and R.F. Miller.
Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55455

Ca^2+ currents and intracellular pH were studied in dissociated
photoreceptors from tiger salamander retinas. Cells were loaded with either Fluo-2
(Ca^2+ indicator) or BCECF (pH indicator) and imaged using a dual wave length
ratios imaging technique. The fluorescence was collected using a cooled CCD camera
(Photometrics). Ca^2+ currents were evoked by introducing high potassium concentration
in the bathing solution for short duration (~5 s).

High potassium evoked Ca^2+ currents were mainly localized
in the photoreceptors soma and pedicle. Elevated of (Ca^2+), was also observed in the
dense layer, and to a lesser extent in the outer segments, of the retina. Ca^2+ current
was slowly suppressed following introduction of Cl^- medium (both SO_4^- or
CHES/NEAT were used as Cl^- replacements). At the same time no change in intracellular
pH was observed. This was detected, introducing NaCl of -30 mV, sufficiently long to
induce intracellular CI^- changes extracellular CI^- acidification (pH 8.0 to 6.0) along
with the high potassium stimulus was sufficient to suppress the Ca^2+ currents,
suggesting an extracellular mode of action of suppression of Ca^2+ currents.

These results point to parallel mechanisms for the effects of pH and
extracellular Cl^- on Ca^2+ currents in tiger salamander rods.

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EY008844)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 26, 1994
INNER SEGMENTS OF SUNFISH SINGLE CONE PHOTORECEPTORS ARE GRADATED INDEX WAVEGUIDES: M. P. Rowe, N. Engheta, S. S. Ester Jr., J. M. Corcoba and E. N. Pugh Jr. Inst. of Neur. Sci., Univ. of PA, Philadelphia, PA 19104; Dept. Elecr. Eng., Univ. of PA, Philadelphia, PA 19104; Dept. Bio., Univ. of Mich., Ann Arbor MI 48109; Deps. Cell Bio. Neurobio., & Ophth., Duke Univ. Med. Center, Durham, NC 27710; Dept. Psych., Univ. of PA, Philadelphia, PA 19104 Cone cells isolated from the retina of the green sunfish (Leptosom cyanesse) were examined with a double beam scanning interferometer and also with transmission electron microscopy. With the interferometer, optical path lengths (OPLs) were measured perpendicular to the natural direction of propagation of light in the living fish eye. Measurements gave a resolution of 220 nm in diameter by 5 nm in path length for 632.8 nm (free space) incident radiation. By assuming cylindrical symmetry for the inner segment region of the cone cells, variations in refractive index can be inferred from these OPL measurements. Our analysis indicates that the average refractive index increases monotonically from around 1.39 at the base of the inner segment to 1.45 near its junction with the outer segment. This increase is consistent with general predictions based on an analysis of electron micrographs previously presented (Rowe et al., J. Optical Soc. A, 11:55-70).

SPECTRAL SENSITIVITY OF RETINAL CONES IN DANIO MALABARICUS (Giant danio): A. G. Palacios, B. Silvestrini & T. H. Goldsmith. Department of Biology, Yale University, New Haven, CT, USA.
The spectral sensitivity of individual cones of a fresh water fish (Danio malabaricus, Cyprinidae) was measured between 280 and 700 nm by recording membrane potentials with suction electrodes in response to brief flashes of monochromatic light of known quantal flux. These measurements extend further into the UV than have previously been obtained by this technique. We found four classes of cones with maximal sensitivity at 360 (n=5), 406 (n=7), 480 (n=10) and 561 (n=4) nm. With the exception of the UV-sensitive cone, all spectral sensitivity curves were well fit by an isopolar template.
The identity of the retinoid chromophore was established by HPLC. Retinal oximes were recovered from eyecups by extracting with a 1:1 mixture of methanol and 1M hydroxyamine (pH 6.5). The oximes were separated on a normal-phase silica column developed with 9% dimethane in hexane. Individual eyes contained an average of 580 pmol of retinal and 10 pmol of 3-dehydroretinal. Supported by NEI grant EY00222

HUMAN CONE ELECTRORETINOGRAM CAUGHT BY SECOND-PHASE CURRENTS: M. Sasaki, P. Sugita* & M. Ikeda.* P. Iachellope. Department Ophthalmology, Columbia University, NYC 10032 & McGill University, Department Ophthalmology, Montreal, Canada

The b-wave of the cone electroretinogram (ERG) of monkeys and man to a flash can be caught and reduced in amplitude by a second flash if it occurs within 20 ms after the first flash. The reduction is maximum at 10-15 msec after the first flash. It occurs at different states of retina adaptation, flash intensities and rates of double flash presentation. We suggest that this interaction between double flashes may reflect timing differences in the cone to on-off and bipolar synapses (Ashmore & Falk, 1980; Copenhagen et al, 1983) that lead to the generation of opposing ionic currents (Sieving et al., 1994) which underly this retinal response.

Supported by NIH Grant EY04138, National RP Foundation, Research to Prevent Blindness, Inc.


Introduction: Rod and cone signals in amphibian retina may interact at the level of the photoreceptors themselves and on second order cells. We have examined the role of D, dopamine receptors in modulation of both rod-cone coupling and transmission between Xenopus photoreceptors and L-horizontal/bipolar cells. Results: a null test, based on equal rod absorbance for red and green flicker indicated a significant presence of cone signal in mesopic and photopic rods. EM showed small gap junctions between rods and cones and injection of neurobiotin into rods labeled both adjacent rods and cones (ratio 1:1). The magnitude of cone input to rods could be manipulated with D3 agonists/antagonists. The flicker fusion frequency of dark adapted rods (4-5Hz) was increased by the D3 agonist, quipazine (10-10 M) to 12-20 Hz. This effect was observed with red, but not green flicker, its magnitude depended on the intensity and it was not affected by SKF 38393, CNQX or kynurenic acid. Spiperone, a D3 antagonist, reduced v to 2-3Hz. In the dark adapted retina the cone signal was absent from the HCs, but was expressed strongly in bipolar cells, as evidenced by intracellular recording and ERG. Cone input to bipolar cells was blocked by the D3 antagonist and increased by the D3 agonist. Conclusion: Transmission of cone signals to rods and to bipolar cells is modulated by a D3 dopamine mechanism. In the dark adapted state, cone signal continues to be expressed in the vertical (bipolar cells) pathway as a result of D3-dependent modulation, but is suppressed in the horizontal pathway. Acknowledgment: supported by EY 0570 to P.W.

ELECTRICAL RESONANCE OF FROG SACULAR HAIR CELLS MEASURED IN THE PERFORATED-PATCH AND WHOLE-CELL RECORDING CONFIGURATIONS: D. Leventhal* and W. M. Roberts. Institute of Neuroscience, University of Oregon, Eugene, Oregon, 97403

Frequency discrimination in the inner ears of birds, reptiles and amphibians is accomplished in part by the electrical resonance issue to mechanoreceptive hair cells. Saccular hair cells of the frog (Rana pipiens) express a damped membrane potential (Vm) oscillation caused in part by the interactions of voltage-gated Ca channels with their actin-based active zones. Since an abundant, mobile Ca buffer appears to shape the Ca landscape at active zones, we asked whether its presence (in perforated-patch recordings, PPRs) interfered with Vm oscillations (in whole cell recordings, WCRs, where the native buffer was replaced by 1 mm EGTA) induced Vm oscillations recorded in current clamp. Oscillations during and after current steps were fitted with a damped sine wave by a least-squared-error algorithm at measure frequency (f), damping time constant, and quality factor (Q) oscillations in 7 cells in each recording configuration. Zero-current potentials were -53.68 mV (mean ±5.9 mV) in PP (series resistance, Rs = ±33.3 MΩ), and 40.2 mV in WC (Rs = 3.3 ±1.6 MΩ) measured at 90 ms after break-in. The relationship between Q and Vm was bell shaped; peak Q (where the resonance was most robust) was 10.6 ±6.7 in PP, and 8.9 ±1.0 in WC. The potential at peak Q was 2.5 ± Mv positive the zero-current potential in PP, and +6.12 mV in WC. The f measured at peak Q was 19.8 ±21.2 Hz in PP, and 2130 ±26.8 Hz in WC. Comparisons between peak Q, f at peak Q, and zero-current potentials in PP and WC were not significantly different using the Mann-Whitney test. These results suggest that the mobile Ca buffer does not greatly affect the resonant properties of frog saccular hair cells. Supported by NIH grant NS 27142 and a McKnight Scholar Award to WMK.

TWO TYPES OF INWARD RECTIFIERS IN HAIR CELLS OF THE MOUSE ULTRICLE A. Rossire & R. A. Fantook. Dept. of Otolaryngology, Baylor College of Medicine, Houston, TX 77030.

Whole-cell currents were recorded with the patch-clamp technique from hair cells in utricles excised from 2 to 17 d old mice. Recordings were obtained from both type I and II hair cells, the two morphologically classes of hair cells in mammalian vestibular organs. Two kinds of inward rectifying conductances were found. One type, resembling the inward rectifier /k1/, activated negatively to -50 mV with fast monoeponential kinetics. Its mean chord conductance at -124 mV was 3.3±1.1 nS (mean±SD, n=8 cells). It was blocked by 1 mM external Ba2+ or Ca2+.

The second type activated more slowly and with a sigmoidal time course at potentials negative to -60 mV. Its maximal conductance, expressed as a first-order Boltzmann relation with a V0.5 = 101.5±7.3 mV. The reversal potential was 43±1 mV (n=6 cells), and the chord conductance at -124 mV was 1.7±0.3 nS (n=8 cells). This current persisted in 1 mM external Ba2+ or 4 AP or intracellular Ca2+, but was blocked by 1 mM external Ca2+. This current is similar to /k1/ of photoreceptors and cardiac cells.

/k1/ was found in 24 out of 48 cells, including both type I and II. /k1/ occurred in most type II cells, including cells with /k1/ but we have not yet determined whether /k1/ occurs in type I hair cells.

(Supported by NIH grant DC02290)
K CURRENTS IN TYPE I AND TYPE II HAIR CELLS OF THE RAT UTRICLE. M. Shecklard R.A. Fareed, Physiology Dept., Univ. of Rochester, Otolaryngology Dept., Baylor Coll. of Med., Houston, TX 77030.

Whole-cell currents in rat utricular hair cells were studied with the ruptured- or perforated-patch technique. Cells with constricted necks were identified as type II. A low-voltage-activated K current (IK) was correlated with type I cells: 95 and 2% of type I and type II cells, respectively, had IK. IK was activated above -95 mV and saturated by -30 mV. Boltzmann fit to the activation curve gave a slope (k) of 5.60 ± 2.0 mV (SEM, n = 29) and a half-maximal voltage (V1/2) that varied between 55 and -73 mV. Because IK1 was substantially on the zero current potential (V = -724 ± 7 mV, n = 37), type I cells had lower input resistances and faster responses to input currents than type II cells (V = -648 ± 2 mV). The distributions of input resistances at -64 mV of cells with and without IK had modes of 21 MQ (n = 24) and 3.6 GG (n = 40), respectively. Inactivation of IK was slow and sigmoidal. It was fit by an equation for a 3-state model (2 closed, 1 open), giving t half of -150 and 30 ms at -64 mV. Type II cells had outwardly rectifying K currents that activated above -60 mV and had variable activation and inactivation kinetics. Monoexponential fits to inactivating currents at -40 mV produced t's that varied from 20 ms to 700 ms. The data are consistent with the cells having, in different proportions, two outwardly rectifying conductances with distinct kinetics. The cells with the slowest currents may have just one current; their activation curves were fit with a Boltzmann function with a V1/2 of -175 mV and an r value of 86.1 mV (n = 14). Ca2+-dependent K currents in type II cells were negligible, even with the perforated-patch method. (Supported by NIH grant DC02590.)

CAULCIM-ACTIVATED POTASSIUM CHANNELS OF TURTLE COCHELAR HAIR CELLS. L. J. Ayer, Y. C. Wu and R. Fettiglasse. Department of Neurophysiology, University of Wisconsin, Madison, WI 53706 and Department of Pharmacology and Physiological Sciences, University of Chicago, IL 60637.

A major factor determining the electrical resonant frequency of turtle cochlear hair cells in the range 50-500 Hz is the time course of the Ca-activated K current. We have examined the notion that this time course is dictated by recording Ca-activated K channels in inside-out patches from isolated cells. A cell's resonant frequency was estimated from its known correlation with the dimensions of the hair bundle. All cells that possess BK channels with a similar unit conductance (300 pS in symmetrical 150 mM K) but with different mean open times of 0.25-12 ms. The time constant of relaxation of this Ca-activated single channel conductance (50 mV, 4.5 μA) varied between cells from 0.4 to 13 ms and was well-correlated with the hair bundle height. The magnitude and voltage-dependence of the time constant agree with the expected behavior of the macroscopic K current, whose speed is thus limited by the K channel kinetics. All BK channels had similar Ca sensitivities with a mean half-activation at 5 μM (0 μM); we estimate that under physiological conditions these channels may be completely activated by a local rise in Ca to 50 μM. Membrane patches also contained 30 pS SK channels which were about ten times more sensitive than BK channels. At -50 mV, Charybdotoxin (0.1 μM) blocked both types of K channel. The SK channels may underlie the hair cell's different isoforms. (Supported by NIH grants DC02054 (L.J.A), and DC01352 (R.F.)}

MOLECULAR CHARACTERIZATION OF A VOLTAGE-ACTIVATED CALCIUM CHANNEL FROM THE CHICKEN'S INNER EAR. R. Kollmar and A. L. Hudspeth. Howard Hughes Medical Institute and Center for Basic Neuroscience Research, University of Texas Southwestern Medical Center, Dallas, TX 75325.

The influx of Ca2+ into hair cells of the inner ear is necessary both for frequency tuning by electrical resonance and for synaptic transmission to auditory neurons. Unlike most other voltage-activated, L-type Ca2+ channels, those of hair cells are not voltage dependent. Membrane potential are not inactivated by Ca2+ influx. To understand the molecular basis of these unique characteristics, we have begun to determine the structure of Ca2+ channels from the chicken's inner ear.

Using RNA from the sensory epithelium of the basilar papilla and populations of hair cells with degenerating hair bundles, we obtained over 5 kilobases of cDNA, lacking only the 5' end, for the α1 subunit of a voltage-activated Ca2+ channel. The predicted protein sequence of the four homologous L-type Ca2+ channels is more than 90% identical to those of mammalian L-type channels of class D; however, several short stretches diverge markedly from any published sequence. We have observed additional diversity at the 3' end; two classes of isolated cDNAs encode alternative cytoplasmic domains at the carboxy termini, neither of which resembles published sequences. We are now testing whether these divergent sequences define new functional subtypes that account for the unusual electrophysiological properties of the voltage-activated Ca2+ channels there. This work was supported by NIH grant DC00317.


The frequency of electrical resonance in turtle auditory hair cells in the range 5 to 300 Hz is correlated with the kinetics of the cell's K current. Using whole-cell voltage-clamp recordings, we have examined the extent to which these kinetics are determined by the relative contributions of distinct conductances, with different pharmacology, to calcium- and voltage-sensitivity. In high frequency cells (>200 Hz), superfusion with TEA and 4-AP block the K current with affinities of ≏200 μM and ≏10 μM, respectively. This current is also blocked by the selective di- and trivalent cation, K+ (Ca2+ > Mg2+ > Na+). In low frequency cells (<50 Hz), the K current is sensitive to 4-AP (K+ ≏25 μM). This current is resistant to lowering [Ca2+] or intracellular perfusion with 30 mM BAPTA. It activates less steeply with voltage, and the current is also consistent with a smaller unitary conductance. A selective inwardly rectifying current is also present. Cells tuned to intermediate frequencies exhibit a mixture of both 4-AP- and TEA-sensitive K currents. Across the frequency spectrum, an additional, small, voltage-independent K current is present to a variable degree, and it is most easily observed under conditions that elevate [Ca2+]. We propose that this current carries underlying resonant behavior that is systematic in the auditory epithelium and solitary cells that resonate at the lowest frequencies employ an ensemble of K currents distinct from those characterized previously for resonant frequencies greater than 50 Hz.

Support: HHMI Predoctoral Fellowship (MBG) & NIH grant DC054 (JFA).

CALCIUM REGULATION IN TURTLE COCHLEAR HAIR CELLS. T. Tucker and R. Fettiglasse. Department of Neurophysiology and Neuroscience Training Program, University of Wisconsin, Madison, WI 53706.

The large size (up to 0.1 na/f) of the sustained Ca current in turtle hair cells imposes a considerable burden on the cell's Ca regulation about which little is known. Whole-cell recording of Ca currents from isolated hair cells using cesium-filled electrodes revealed a slow inward component of the tail current which declined over 0.2 to 2 s. The size of the tail current increased with the duration of the preceding voltage-step, exhibited a dependence on membrane potential that paralleled the Ca current and, like the Ca current, was blocked by 20 μM nifedipine. The tail current was abolished by removal of external Ca, but not by removal of Na or Cl. These properties argue that the current is carried through Ca-activated K channels, its time course reflecting the decline in Ca concentration at the membrane. Thapsigargin (0.2 μM), a specific blocker of the endoplasmic reticulum Ca ATPase, and intracellular vanadate (1 mM) caused a progressive increase in the tail current on consecutive voltage pulses culminating in a permanent activation of an inward K current, which suggests the Ca load was no longer being cleared. We conclude that a Ca ATPase, probably located in an intracellular compartment, plays a major role in the Ca regulation in turtle hair cells and speculate that Ca may eventually be extruded by fusion of the stores with the plasma membrane.

MOLECULAR IDENTITY OF A POSSIBLE HAIR-CELL MYOSIN I. A. B. Metcalfe and A. J. Hudspeth. Howard Hughes Medical Institute and Center for Basic Neuroscience Research, University of Texas Southwestern Medical Center, Dallas, TX 75325-9117.

The hair cell's mechanosensitive organelle is the hair bundle, an organ-pipe array of stereocilia. A tip link connects stereocilia at the tallest neighboring process; at or near this connection lie transduction channels that are gated by bundle displacements. During a hair cell's adaptation to protraction or retraction, the current flowing through these transduction channels returns toward its resting level. Adaptation is thought to involve adjustment of the tension within tip links by an ATP-requiring molecular acceptor. The tension is regulated by the myosin isoforms. Given this hypothesis, monoclonal antibodies raised against a bovine myosin I, MMB, recognize a 120-kDa protein from hair bundles of the frog's sacculus. Immunofluorescence microscopy reveals that this protein is concentrated at the bundles' top edges, where the tip links and transduction channels are located. To determine the molecular identity of the myosin isozone in hair bundles, we have investigated expression in the frog of a gene similar to that encoding MMB. Frog brain, which contains a 120-kDa protein recognized by the same antibodies, expresses an mRNA that is closely resembles that of MMB message. The sacculus macula contains mRNA with a similar sequence. When expressed in bacteria, a portion of the sequence specifies a protein that is recognized by antibodies raised against MMB. We are now attempting to determine the full-length sequence of the myosin message from frog brain. This work was supported by NIH grant DC00241.

An acidic amino acid, such as glutamate, has been implicated as the hair cell transmitter in anamniotes (e.g., frogs), which lack Type I hair cells. Since there is scant electrophysiological evidence of the nature of the hair cell transmitter(s) in anamniotes, we have begun to investigate if glutamate is the transmitter from canal afferents in the turtle inner ear (N-9). Bath-applying transmitter agonists and antagonists in order to relate their activity with the endogenous hair cell transmitter-mediated activity. During extracellular recordings from individual afferents, glutamate (5 mM) reversibly induced an increase in the firing frequency of the afferents (N=29). Aspartate (5 mM) mimicked this effect (N=5). Kynurenic acid (1 mM) reversibly eliminated the resting firing frequency of the afferents (N=8) and reduced the excitatory action of both of these agonists (N=6). NDMA (0.5 and 5 mM) also resulted in afferent suppression (N=5). GABA (5 mM) had no consistent effect upon afferent firing (N=10), while carbachol (0.5 mM) resulted in an increase in firing, weaker than that induced by glutamate and which to some extent desensitized during application (N=6). These findings are consistent with the hypothesis that glutamate or a related compound is a hair cell transmitter in anamniotes as well as anamniotes. The major difference between the turtle and the frog appears to be the sensitivity in the turtle to NDMA, not found in the frog. Otherwise the afferent response resemble those found in afferents innervated by Type II hair cells in the frog. It is as yet unclear as to whether or not axons innervated by Type I hair cells are also included in the current sample. Supported by NASA grant NAG 2-780 to SLC and NIH DC-01273 to MJC.

398.11 LOCALIZATION OF NITRIC OXIDE SYNTHASE IMMUNOREACTIVITY AND NICOTINAMIDE ADENINE DINUCLTEOTIDE PHOSPHATE DIAPHORASE IN THE RAT INNER EAR. M. Lyon, B. Godri and B. Mayer, 1 Dept. Otolaryngology, SUNY Health Science Ctr, Syracuse, NY; 2 Inst. Pharmacology Toxicology Univ. Graz, Graz Austria.

Nitric oxide (NO) has received considerable attention and appears to play critical roles in neurotransmission. There is evidence showing that NO can function as a neurotransmitter (Bredt et al., Nature 351:714; 1991); is involved in ischemic injury (Beckman, Nature 345:27; 1990); plays a role in regulation of cerebral blood flow (Iadecola, Prot Natl Acad Sci USA 90:4561; 1992). To determine if NO may be involved in inner ear function, a combination of histochemical and immunohistochemical techniques were used (Mayer et al., FEBS Lett 277:215; 1990) to localize nitric oxide synthase immunoreactivity (NOS) within the adult rat temporal bone. Preliminary results show similar patterns of labeling for both techniques. Scarp and Scarp's ganglion cells are positive as are their nerve projections. Label is also observed within the perineuropil of all the vestibular end organs with dense label near base of some hair cells bases. The stria vascularis contained NADPH-diaphorase label but little NOS immunoreactivity. These results indicated that NO may play a role in inner ear function and that a different or previously undescribed type of NOS is located in stria vascularis.

398.12 THE EXPRESSION OF GROWTH FACTOR RECEPTOR GENES IN MAMMALIAN HAIR CELL EPITHELIUM ASSESSED BY RT-PCR. L.D. Saffert and J.J. Currie. 1 Dept. Otolaryngology and Neurosci., Univ. of Virginia, Charlottesville, VA 22908.

Hair cell loss in the vestibular organs of mammals has been shown to result from the proliferation of progenitor cells, which are the progenitors of regenerated hair cells. In normal cell proliferation, nitrous growth factors must bind to specific receptors before cell division can occur. Since we are interested in controlling hair cell regeneration, we need to identify the receptors that are expressed in these cells. RT-PCR provides a sensitive method to assay for the presence of low levels of messenger RNA for such receptors. Hair cell epithelia were isolated from undamaged uroticules of rats, trimmed of all edges and extracellular ameila in liquid nitrogen. Total RNA was isolated from these tissues and reverse transcribed into intronless cDNAs. RT-PCR primers were selected from published sequences for growth factor receptors and these were used in hot-start PCR to amplify unique fragments of receptor cDNAs. Positive and negative controls were run with each reaction. PCR products were resolved on gel electrophoresis. These experiments have demonstrated the presence of mRNA for the insulin receptor, but as yet have not shown detection levels of mRNA for the PDGF receptor, the IGF-1 receptor, or the EGF receptor in central regions of undamaged hair cell epithelia from mammalian utricles. Undamaged uroticules will be assayed for increased expression of those messages.

398.13 THE PROLIFERATION OF HAIR CELL PROGENITORS IS DEPENDENT ON CELL-SUBSTRATE ADHESION. L.L. Cunningham, J.E. Finley, M.S. Norgol, and J.T. Corwin, Dept of Otolaryngology and Neurosci., Univ. of Virginia, Charlottesville, VA 22908.

We conducted experiments to determine whether adhesion to a substrate is required for the proliferation of supporting cells from the chick ear. Basilar papillae and strabyles were dissected from young (7-14 days) white leghorn chickens, and the sensory epithelia were cultured for 5 days in Medium 199 supplemented by 20% fetal bovine serum. After 1-2 days in culture, hair cells extruded from the epithelium leaving sheets of supporting cells. A subset of the supporting cell sheets attached to the substrate and began proliferating. Other sheets continued to float in the culture medium during the same period. Bromodeoxyuridine (BrdU) was added to the culture medium for 4 hours to measure the proliferation rates of substrate-attached supporting cell sheets. BrdU-positive sheets were counted. Twelve adherent cultures had a mean of 29.2 ± 3.4 BrdU labelled cells per 100,000 µm² (range; 8-59). Whereas nine free-floating cultures had a mean of 4.5 ± 0.5 BrdU labelled cells per 100,000 µm² (range; 0-24), a significantly (p > 0.005) lower proliferation rate. The supporting cells were in the presence of the many mitogenic growth factors in serum, but only showed a significant proliferation after substrate adhesion, suggesting that adhesion is required for the proliferation of auditory and vestibular supporting cells. Preliminary immunohistochemical studies reveal the presence of integrin receptors on the supporting cells which may be responsible for the adhesion-dependency of their proliferation.

398.14 CYCLIC AMP IN AN INNER EAR HAIR CELL SHEET PREPARATION. M. J. Dreger, D. J. McGinley, and D. G. Dreger, 1 Lab. of Bio-chem. Dept. of Otolaryngology and 2 Bioch. in the 3 Univ. of Iowa, 2014, 1991.

Previously, we localized adenylyl cyclase activity to specific sites within the sensory epithelia of the neural and non-neural macaqueauditory hair cell. Precipitate was also found in two groups of nerve terminal in contact with hair cells. This paper describes the distribution of adenylyl cyclase activity and its potential significance in the development, maintenance and regeneration of macaque auditory hair cells. We have also shown that cyclic AMP can be a mitogenic agent for hair cells.

With an enzyme immunoassay (ELISA) for cAMP (Amersham, RPN 225), we have now measured cAMP levels in a hair cell sheet preparation for which the hair cell is the only intact cell type. Pseudolaric acid extracts of the hair cell sheet and associated vascular nerve fibers contained 5.0±2.1 fmol cAMP/mg cell protein, respectively [mean ± SEM (n)]. Depolarization of the hair cell sheet by a HEPES-containing medium and 100 mM KCl floating sheets increased cAMP levels to 10 fmol/1000 cells, compared to 1 fmol for glial cells, the latter presumed to be involved in receptor activation. Extracellular application of cAMP by microinjection experiments induced an increase in influx of cAMP of approximately 0.6 fmol/7000 hair cells, compared to 1 fmol for glial cells, the latter presumed to be involved in receptor activation. Extracellular application of cAMP at 25 µM (5 min) was observed to elicit a 30° increase in intracellular levels of cAMP in the hair cell sheet relative to the paired control, significant by paired-variate analysis (p < 0.05, two-tailed test). The low level of cAMP in the hair cell sheet appears consistent with its limited and site-specific presence in the cochlear lateral wall, and the absence of cAMP synthesis. The demonstration of intracellular cAMP in the hair cell sheet by extracellular ATP, if not neglected with regard to mechanism, represents preliminary evidence of the existence of a cAMP-mediated second messenger pathway in hair cells.

(Supported by Deaf. Res. Found. [MDI] and NIH [DC00156, DC00026])

The functional polarization vectors derived from otolith primary afferents are commonly fit to a cosine. The morphological polarization vectors (MPV) of hair cells contribute to the afferent's response characteristics. In addition, examination electron microscopy of rat utricles and saccule demonstrates that adjacent hair cells often have MPVs that vary significantly. Physiological studies of individual hair cells have demonstrated that the functional polarization vector is not described by a cosine. Instead, the differential gains for the positive and negative half cycles are not equal and produce a clipped response waveform. We believe that this is a necessary account for the deviation from the cosine relationship found by Fernández, et al. (J. Neurophysiol. 35, 1972) in their studies of otolith responses. We have simulated the effects of hair cell MPVs on the resultant afferent polarization vectors using a simple representation for the nonlinear hair cell input/output characteristics. Each afferent using this model exhibits the same type of nonlinearity found in otolith afferent fibers. These findings are unchanged when the contributing hair cells have widely varying MPVs. A comparison of the simulation results with otolithic response characteristics suggests that the property responses of otolithic afferent fibers is consistent with relatively simple integration of input from contributing hair cells. The attendant nonlinearity in the responses results from the input/output characteristics of the hair cells.

This work was supported by NASA and the National Academy of Sciences.


Aldosterone, a mineralocorticoid, enhances Na retention and K excretion in various ion transporting epithelia. Previous studies have demonstrated that this mineralocorticoid regulates Na, K-ATPase in the inner ear. Na, K-ATPase has been implicated in the maintenance of high K concentration in endolymph, ion transport processes which support transduction by inner ear hair cells. Other studies have revealed a similar pattern of distribution for mineralocorticoids in the (type II) binding sites and Na, K-ATPase. These results indicate that the mineralocorticoids may regulate endolymphic ion content and thus modulate hair cell transduction. The purpose of our present study was to determine if mineralocorticoid binding sites are associated with hair cells of the cristae ampullare.

The ampullae of the posterior, lateral and superior semicircular canals were dissected from male Hartley guinea pigs and incubated in media containing ([1H]-aldosterone (30 nM) and a 1000-fold excess of unlabeled aldosterone. Responsive sites were then processed for autoradiographic analysis.

Examination of autoradiographs revealed silver grains in the sensory epithelium of the ampullae. Specifically, binding was localized in the nucleus, cytoplasm, and stereocilia of both type I and type II hair cells. Localization of mineralocorticoid binding sites to these cellular components suggests that aldosterone may participate in both growth-related and non-specific modulation of hair cell transduction.

(Marked by NIH CDA DC0006 and D.Z.P. and NIH T32 DC0038)


A piezoelectric micro-actuator was used to generate mechanical indentations of the long and slender region of the horizontal canal (HC) and the utricle (U) in the toadfish, Opsanus tau, while recording extracellular afferent responses. In the toadfish, the HC and U were indented in a linearly varying (±16 μm) indentation at 2.4 degrees/sec. Head motion. Having established this correspondence, step mechanical indentation was applied to stimulate a step increase in head velocity (Dickman and Korn, J. Physiol. 162, 1962). Aortic responses showed a jump in pressure (±0.02 sec) in firing rate followed by a fast transient decay (±1 sec) and a slow exponential decay (±30 sec) approximating the resting rate. The slow exponential tail decay across units and is consistent with models describing the mechanical recovery of the cupula to its original resting position. The jump increase and fast transient decay show significant inter-unit variability, from being absent in some units to exceeding the peak magnitude of the slow response in others. This fast component cannot be described by models of macromolecular cellular displacement or by established processing associated with the hair-cell-afferent complexes. The range of afferent responses to head rotation can be described by a model in which the stimulus transfers to macromolecular endolymph flow and pressure combine within the structural mechanics of stereocilia, although the macromechanical and/or biophysical mechanisms responsible for the apparent unit-specific sensitivities to these components remain yet unknown. [Supported by NIH]

TOULENE EFFECTS ON Gullus domesticus' VESTIBULAR SENSORY EPITHELIUM IN THE EMBRYOGENESIS. A. Illescas-Landrace and M. Lorenzo-Illanes-Monje. Departamento de Anatomo y Departamento de Farmacologia. UNAM, Mexico, D. F. 04510.

All biologically active substances interfere, in one way or another, with the chemical system that governs the homeostatic mechanism of living organism. Many drugs create temporary or permanent alterations in auditory or vestibular function.

The purpose of this study is to show that toluene induces cellular alterations on Gullus domesticus internal ear vestibular sensory epithelium during its morphogenesis. To accomplish this purpose 42 fertile stage 6 (23-25 mm) zebrafish were used arranged on 7 groups. The applied toluene doses were 0.60, 1.12, 2.5, 5.0 and 10.0 μL for the first 5 groups; 10.0 μL of normal saline solution for the 6th group and the last one was taken as a control group. After 14 days of hatching the embryos were removed from their egg-shells. Immediately were bled and their internal ear were prepared to be analysed through electron microscopy. Toluene provoked loss of some hair cells in all cristae and otolithic organs, in other cells we found various stages of degeneration: loss of cell, cytoplasmic degeneration and vacuolization of membranes. In the supporting cells the common damage observed was edema. These data suggest that toluene injured avian vestibular receptors during the embryogenesis.

RAPID DENDRITIC GROWTH IN VESTIBULAR NEURONS OF PERINATAL CHICKEN. G. C. DeRubertis. Biocomputation Laboratories, University of Washington, Seattle, WA 98195.

The objective of this study was to determine whether perinatal remodeling of primary vestibular synapses in the medulla is accompanied by developmental changes in their target vestibular sensory neurons; the pricipal cells (PC) of the tangential nucleus. PCs are vestibularocollateral collic neurons which receive large axosomatic "spoon" endings from primary vestibular fibers. Spoon synaptogenesis follows an unusual pattern: first in embryos (E7) forming chemo-synapses, with the growth cones of the inner hair cells. These spiny zones (Gzs) to form simple synapses (E15), and finally mainly Gzs in hatchlings (H), P. Neurol. 230:389-392 (1994).

This is the first report documenting PC dendritic morphology and comparing them at two ages, E15-16 and H-2. From preliminary observations made on PCs intracellularly injected with biocytin, we observed that the characteristic oval somata do not change appreciably in size (28±5 SD x 18±3 μm; n=12 vs. 26±4 x 17±5 μm; n=9). Also, in our small sample there is no apparent change in the average number of primary dendrites (3.7 dendrite/soneuron) nor in the dendritic branching pattern. However, the number of dendritic branch points per neuron increases by 52% (12.7 ± 1.6) as well as the dendritic spread in both the medio lateral (98%, 168±57 μm vs. 333±76 μm and the dorsoventral axes (127% 103±59 μm vs. 234±71 μm). Finally, a major change of 147% was observed in total dendritic length per neuron (605±26 μm vs. 1495±35 μm).

These observations suggest that parallel to the important changes occurring at spoon ending terminals at hatching, their postnatal target cells rapidly and extensively generate new dendrites. (This work was supported by NIH grant RO1-DC00970).
399.1


Stebler and Narins (1990) reported that increasing body temperature causes low-frequency auditory nerve fibers in the frog to increase their CFs and decrease their thresholds. We have previously identified two-tone squeeze suppression (TRSS) and excitatory tuning in the frog, and we now report findings that suggest TRSS may be dependent on the temperature. Squeezed suppression might be expected to exhibit similar temperature-induced shifts.

The goal of the present study was to examine the temperature dependence of auditory fibers in the frogs. We found that the frog's response to a two-tone stimulus changed with temperature. The temperature-induced changes in the frog's response suggest that thermal perturbation may affect the frog's response to a two-tone stimulus in a manner similar to that of TRSS in the frog. The temperature-induced changes in the frog's response are likely mediated by changes in the frog's C-fiber activity.

399.2

SIZE, DISTRIBUTION, AND THEORETICAL CONDUCTION VELOCITIES OF AUDITORY NERVE FIBERS IN THE FROG INNER EAR. B. Pesaran, D.D. Simmons, P.M. Narins, S. Parham, C. Bertolotto, and A. Newman. Faculty of Biology and Biophysics, UCLA, and the House Ear Institute, Los Angeles, CA 90024.

The leopard frog (Rana pipiens pipiens) has two specialized auditory organs: the amphibian papilla (AP) and the basilar papilla (BP). In this study, we used two-tone suppression to study the effects of cooling the fibers in the frog with a temperature range of 1-15°C. The results suggest that the frog's response to a two-tone stimulus is largely determined by the temperature. The frog's response to a two-tone stimulus is largely independent of the temperature, indicating that the frog's response is temperature-independent.

399.3


We previously demonstrated that fibronectin-like immunoreactivity is depressed following noise-induced temporary threshold shifts (TTS). We have now examined the depression of E1 class immunoreactivity, a marker of integrin localization in the cochlea during TTS and subsequent recovery. We observed a significant increase at 10-min intervals following noise exposure, which was not observed in the control cochlea. This result implies that E1 class immunoreactivity is depressed following noise exposure and may play a role in the process of recovery.

399.4


Early results imply that 211-2 DPOAEs are produced in frequency regions between the primary tones, while 221-11 DPOAEs are produced at the second tone. These results suggest that 211-2 DPOAEs are produced in frequency regions between the primary tones, while 221-11 DPOAEs are produced at the second tone.

399.5


The cloning of cDNAs encoding two water channels from rat kidney has been recently reported by Deen et al. This work is significant for the following reasons: the water channels are expressed in the kidney, and the water channels are expressed in the cochlea. The results of this study support the hypothesis that the water channels regulate the osmotic state of the cochlea.

399.6

INNER HAIR CELLS IN THE COCHLEA OF THE FETAL BRONX WALTERZ MOUSE. D.S. Whitlock*, D. Cahill and M. Zhang. Walter Reed Army Medical Center, University of Wisconsin, Madison, WI 53706.

In the adult Bronx Walterz mouse, more than 75% of the normal number of inner hair cells is missing. The specific role of inner hair cells in the cochlea remains unknown. The Bronx Walterz mouse is a valuable tool for studying the effects of noise and other environmental factors on the inner hair cells.

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399. 7


Published evidence indicates that dopamine is present in the auditory inner ear. Presynaptic dense-core vesicles have been observed in olivocochlear fibers by electron microscopy, and dopamine has been detected at femtomole levels in the rat cochlea. Immunocytochemical studies of olivocochlear fibers in the cochlea and neurons in the lateral superior olivary complex have suggested the presence of tyrosine hydroxylase and absence of dopamine B-hydroxylase and phenylethanolamine N-methyltransferase. We have utilized polymerase chain reaction (PCR) analysis to identify messages for dopamine receptor subtypes in the mouse cochlea. Specific D1A and D1B receptor primers were designed based on published rat brain sequences, and D2, D3, and D4 subtypes from the corresponding mouse brain sequences. Poly A RNA was isolated from total RNA in guinea thycape extracts of cochleas of 30 CB6 mice (16 days old) and reverse-transcribed with oligo dT primer. PCR-amplified products were separated by agarose gel electrophoresis. Results were compared to those obtained without reverse transcription (negative control) and to those using mouse brain mRNA with reverse transcription (positive control).

PCR primers for all of the dopamine receptor subtypes yielded bands of expected molecular mass with cDNA from mouse brain samples, indicating that the primers were functioning. The primer designed to detect the D2 long form produced a PCR amplification product of the predicted size from mouse cochlear cDNA. D1B, D3, and D4 primers yielded faint bands after PCR amplification. The D1A amplification product was not observed. These results suggest that dopamine has a neurotransmitter role in the cochlea. (Supported by NIH Grants R01 DC01515 and T32 DC00026.)

399. 8


Although many studies have localized receptors postulated to control cochlear blood flow, there is still a dilemma about the exact mechanisms by which these receptors modulate inner ear function. A previous autoradiographic study conducted in our laboratory localized mineralocorticoid receptors in the walls of cochlear vasculature (Sinha et al., Soc. Neurosci. Abstr. 580.2: 1418, 1993). Furthermore, the possible physiological significance of these receptors in the regulation of cochlear blood flow is of importance in the study of the effects of local and systemic mineralocorticoid analogues and their vasodilator properties. In this study, the possible physiological significance of these receptors in the regulation of cochlear blood flow (CoBF) was assessed by the use of D1 or D2 doppler flowmetry (Sinha et al., Assoc. Res. Otolaryngol. Abstr. 13: 303, 1990). In both studies, we postulated that mineralocorticoids participate in regulation of CoBF.

In order to elucidate the mineralocorticoid participation in cochlear homeostasis, male Hartley guinea pigs were infused with aldosterone (10 μg/kg) for 10 min. Continuous measurements of lateral wall capillary blood flow velocity were assessed with intravital microscopy. Blood samples, withdrawn 2 and 60 min following completion of infusion, were analyzed for serum aldosterone and electrolyte values. Preliminary results show that serum aldosterone levels fell significantly after 60 min post-infusion (5200 ng/dl) compared to its 2 min post-infusion levels (731 ng/dl). Systemic blood pressure, serum electrolyte values (Na+, K+ and Cl), and capillary blood velocity were unchanged during the experimental protocol. The stability of capillary blood pressure supports the conclusion of autoregulatory mechanism for CoBF which preserves constant capillary blood perfusion. Future studies, especially the functional effects of such steroids on arterioles (and arterioles) supplying the cochlea, are needed for further delineations the association between mineralocorticoid hormones and cochlear vasculature. (Supported by NIH CIDA DC00046 and NIH T32 DC00026.)

400. 1


The four functional components of the cranial nerves are classified as general somatic and visceral afferents and efferents. Three additional components associated with special senses are also recognized in cranial nerves. These fibers are classified as special visceral afferents (olfactory and gustatory), special visceral efferents (stapedius muscles), and special somatic afferents (visual, auditory, and vestibular). The sensory cells of the vertebrate inner ear are innervated by both vesiculated and nonvesiculated endings are regarded to be invariant in nature. Anatomical studies in all classes of vertebrates have invariably demonstrated that these efferent fibers originate from specific brain stem nuclei. The vesiculated endings form axosomatic synapses with the sensory cells as well as axodendritic synapses with the peripheral processes of the bipolar sensory neurons of the 8th cranial nerve. Physiological data indicate that efferent innervation of the sensory cells of the vertebrate inner ear is either facilitatory or inhibitory in function. Establishment of efferent innervation of the vertebrate inner ear merits a re-evaluation of functional components of the cranial nerves. This report is suggesting that these efferent fibers be classified as “special somatic efferent” component of the cranial nerves.

400. 3

POSTNATAL IMMUNOREACTIVITY AND RETROGRADE LABELING OF OLIVOCOCHLEAR NEURONS IN THE VENTRAL HUMAN BRAINSTEM. D.D. Simmons1, J. Raij-Kubba2, and J.H. King, Dept. of Biology and Brain Research Institute, UCLA, Los Angeles, CA 90024-1606.

The olivocochlear (OC) system is the major efferent pathway to the cochlea and is critical for modulating activity of the cochlear nerve. Acetylcholine and calcitonin gene-related peptide (CGRP) both serve as neurochemical markers for the OC system. Very little is known about the maturation of the immunohistochemical and an in vitro brainstem technique were used. OC neurons were defined by injections into the crossed OC bundle (COB) at the floor of the IV ventricle. During the first postnatal week, all COCB injections resulted in labeled terminals under the COB and labeled cells in the VPO region of the superior olive. Both CHAT and CGRP immunoreactive cell bodies were clearly evident and the labeled cells were within the VPO region of the lateral superior olive. Double labeling of retrogradely labeled neurons. Between postnatal day 0 and postnatal day 8, retrogradely labeled neurons were observed and double labeled with CHAT antibody. The CHAT positive VPO cells were distinct from CHAT positive LSO cells. CHAT positive LSO cells had similar retrograde nature. The majority of retrogradely labeled CHAT and CGRP cells were found either in the medial limb of the LSO or in the capsular regions of the LSO.

Thus, the majority of COCB cell bodies are CHAT positive, CGRP negative, and found within VPO regions. Our results are consistent with the hypothesis that periventricular OC neurons initially terminate on IHCs and then on OHCs before their neurotransmitter expression is mature. (Supported by grants from the NIDCD and the UCLA Academic Senate)

400. 4

THE CYTOARCHITECTURAL ORGANIZATION OF THE MEIDAL OLIVOCOCHLEAR NUCLEUS OF THE GOLDEN J.A. Goebbels1,2, S. Coomeh3, C.A. McCorkich3, and P. Oshel1. 1Farny Hearing Institute and Biology Department, Loyola University of Chicago, IL 60656 and Oberlin College, Oberlin, OH 44074.

The cytoarchitectural of the medial olivocochlear nucleus (MOC), the principal first-order nucleus of lateral line mechanoreceptors, is not well known in any fish. Using Golgi, immunocytochemical and other histological techniques, we recognize at least three layers associated with the MOC. The molecular layer consists of axons from granule cells in the eminens granulair and axons with GABAergic terminals from cells in the mettenreithian nucleus. The granule nucleus consists of dendrites from underlying cerebellar cells extend into the molecular layer, where small, intrinsic neurons are also found. The layer consists of a thick basal dendrite that extends into dense molecular regions, whereas others have 2 or more thin distal dendrites that ramify near the cell body. The deep layer consists of ventral dendrites from both basal and intrinsinc neurons, terminals from primary afferent fibers and commissural GABAergic neurons. The goldfish MOC has a number of organization for the first-order nuclei of other olivocochlear systems and thus, its function may be shaped by similar neural circuits.

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Evidence for Neuromodulators in the Cochlear Nuclei of the Newly Hatched Chick. E.O. Dobkins & G. Laurenti, Division of Biology, 139-74, California Institute of Technology, Pasadena, CA 91125.

Glutamate mediates rapid transmission of auditory information between the VCN and other afferents and the cochlear nuclei. The presence of glutamate (Raman & Trussell, Neuron, 9(92):173) and GABA (Code & Churchill, Hearing Res, 54(91):281) receptors in chick, and of GABAergic terminals in the AVCN, has been demonstrated. The potential contribution of neuromodulators to transmission across these primary synapses is unknown. The existence, and possible co-localization of neuromodulators with GABA and glutamate, in the avian cochlear nuclei has not been investigated. Immunohistochemistry was used to locate somata and terminals containing acetylcholine, catecholamines, and neuromodulators in the cochlear nuclei of the newly hatched chick. Preliminary results suggest that serotonergic and acetylcholinergic cells are not present. Somatostatin is found in terminals in nucleus angularis (NA) but not magnocellularis (NM) or lateralis (NL), and is present in the amygdaloid nucleus. Enkephalin (5-L-enkephalin) is found in somata and terminals of NA. Cholecystokinin was found in somata: NM/NA/NL, and in terminals in NA. Supported by NIMH and the McKnight Foundation.


The SCC (also called juxtagranular) region of the cat AVCN receives preferential input from low spontaneous rate (SR) auditory nerve fibers (Liberman, 1991). Low-SR cochlear ganglion cells are preferentially located on the scala vestibuli (SV) side (Kawase and Liberman, 1992). From these two, we hypothesize that a focal injection of a tracer restricted in the SCC region should label ganglion cells preferentially located on the SV side. The goal of this study is to evaluate this hypothesis. After a 3-10 day survival period following BD injection, the cats were killed. The labeled Golgi-like fibres from the injection site to the ganglion cell soma and their axons were examined. In cases where the injection was centered in the deep AVCN or deep DCN, the labeled cells tended to be evenly distributed on the SV and SC lamans (SF side). In general, the injection site was in the GC/SC region, by contrast, labeled cells in certain ganglion regions were preferentially located on the SV side. This supports the hypothesis. A complicating factor was that there were additional ganglion regions in the latter cases with labeled cells either on the SV or SF side. Also in these cases, label was found in type II ganglion cells and olivocochlear bundle (OCB) fibers in the intraganglionic spiral bundle. This confirms previous findings that the GC/SC region receives input from type II cells and OCB collaterals. [Supported by NIDCD: NH714988.


A monoclonal antibody to synaptophysin, an integral membrane protein found in presynaptic vesicles, was used to immunohistochemically quantify changes in nerve terminal density in the guinea pig cochlear nucleus (CN) 2-161 days after complete unilateral cochlear ablation. In the ipsilateral ventral CN a significant reduction in the density of immunoreactive synaptic profiles was apparent 4-7 days after ablation, presumably corresponding to the loss of cochlear afferents. In the anterior division of AVCN immunoreactive density decreased to 35% of that contralaterally, and in the anterior part of PVCN to 62%. No reduction in immunoreactive density was seen in the deep layer of DCN. After 161 days there was an apparent complete restoration of immunoreactive density in the ventral CN. In PVCN this increased density could be attributed to progressive tissue shrinkage, presumably resulting from loss of cochlear nerve fibers, but in the AVCN tissue shrinkage only partially accounted for the increased density. These results suggest that the initial loss of synaptic terminal density in the AVCN after cochlear ablation may be followed by a partial regeneration at longer survival times. [Supported by DC00199 from NIDCD].


Spontaneous degeneration of the gerbil cochlear nucleus has been shown to be dependent on auditory functional activity. The degenerative lesions primarily affect dendrites while sparing the neuronal cell body. Ligation of the external auditory canal (EAC) in Mongolian gerbils for one week resulted in >30 dB elevation in ABR thresholds at frequencies from 1-52 kHz. This manipulation reduced the area density of a naturally occurring spongiform degeneration in the ipsilateral cochlear nucleus to 30% of that found on the contralateral (unligated) side. Ultrastructural studies showed that some lesions "fill in" with a tubulovesicular material. Aggregations of mitochondria and filaments are also found. The tubulovesicular material is immunoreactive with antibodies to glucose regulated protein-78 (grp78), an ER-resident protein involved in the proper assembly and folding of newly synthesized proteins.


To determine if unilateral cochlear ablation leads to plastic changes in the auditory nuclei, the freshly dissected guinea pig brain stem was cut transversely into 500 μm slices and samples of the LSO, MSO, MNTB, VNLL and IACC were micropunched to measure uptake and release activities. Five days after cochlear ablation, 3H-D-aspartate uptake and release were decreased slightly in the LSO, MSO and VNLL bilaterally, but these activities returned toward control levels at 59 and 145 days. 3H-D-Aspartate uptake and release were increased slightly at 59 and 145 days in the MNTB and IACC bilaterally. 3H-Glycine or 3H-GABA release, unchanged after 5 days except for a bilateral increase in 3H-GABA release in the IACC, increased slightly at 59 and 145 days in the MSO, MNTB, and IACC bilaterally. 3H-Glycine uptake decreased after 5 days, but increased at 59 and 145 days in the LSO and MSO bilaterally. The cochlear nucleus, but not the AVCN, can be lesioned by cochlear ablation and these changes suggest that unilateral cochlear ablation may lead to plastic compensatory responses in auditory brain stem nuclei. (This work was supported by grant DC00199 from NIDCD)
400.11 EXPRESSION OF C-FOS IN THE AUDITORY BRAIN STEM FOLLOWING ELECTROCOCHLEAR INJURY: LOCALIZATION OF THE EZCHELLON OF NAGASU, N.H. Lim, J.M. Miller, and R.A. Altschuler, Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109-0500.

C-Fos expression (as a marker for activation of neurons) was examined in the brain stem of deafened and undeafened rats after electrical stimulation with cochleotopic stimulation (frequency mapping or deafening by inner ear injection of neomycin). After assessment of auditory brain stem response (ABR), cochlear was implanted with electrodes. Either a single ball electrode was placed in the scala tympani of the basal turn of the cochlea or bipolar electrodes were inserted into basal and apical turns. Three days later electrically evoked ABRs were taken, after five days animals were electrically stimulated at three times the EABR threshold for 90 minutes. Thirty minutes after the cessation of stimulation animals were anesthetized and perfused with 4% paraformaldehyde. Vibrissae nuclei were sectioned through the auditory brain stem and sections were immunoreacted with antiserum to C-Fos (Oxxygen Sciences) using ABC Vector Stain immunoperoxidase methods.

In both hearing and deafened animals, many C-Fos immunoreactive (IR) neurons were observed in the ipsilateral and contralateral to the stimulated side in the dorsal cochlear nucleus, nucleus of the lateral lemniscus and the inferior colliculus. The C-Fos IR cells were arranged in band-like patterns in the IC and the contralateral DCN. The number of IR neurons increased with bipolar stimulation. C-Fos IR neurons were occasionally seen in the superior olivary complex in perilobular nuclei and the lateral superior olive. One deafened animal demonstrated many C-Fos IR cells in the ventral cochlear nucleus but this was atypical. These results suggest that C-Fos upregulation may provide a method for assessing central auditory activation with cochlear implants and may be useful for comparing different stimulation paradigms as well as assessing their influence on deafness-related changes.

400.12 CHARACTERIZATION OF MOSSY FIBER PROJECTIONS FROM THE CUNEATE NUCLEUS TO VARIOUS LOCATION OF THE OZUOINURO, N.H. Lim, J.M. Miller, and R.A. Altschuler, Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109-0500.

The cuneate nucleus (CN) which receives somatosensory input from the auditory nerve. Some non-auditory inputs to the CN have also been described, including a somatosensory input from the hypothalamus (Neurosci. 1987; 20:209-219). We have recently shown that the cuneate projections form mossy fiber terminals in the granule cell domains of the dorsal CN (Wright and Ryugo, 1994). Further studies using the anterograde tracer Phoxolar vulgaris, leucocystiolitin (PHA-L) combined with immunofluorescenc have demonstrated that cuneate projections give rise to mossy fiber terminals in granule cell domains throughout the CN. The trajectory of the cuneate fibers is demonstrated in coronal sections of the brainstem where fibers are seen traversing the descending tract of V before turning mediasd to the auditoal sound nucleus along the stria for varying distances before entering the nucleus at different diencephalonic levels, where they give rise to many en passant and terminal boutons in granule cell regions.

Cuneate mossy fiber terminals contain round synaptic vesicles and form asymmetric synapses with their targets, indicating that they are excitatory in nature. Using immunocytochemical techniques, we have begun to investigate which neurotransmitter is used by the cuneate mossy fibers. Preliminary experiments indicate that anterogradely labeled cuneate fibers are not double labeled with an antibody against acetylcholine (Boehringer Mannheims), an enzyme used in the synthesis of acetylcholine. However, in adjacent sections, PHA-L-labeled cuneate fibers coexist with gluatamate immunoreactive fibers. We will test the hypothesis that the cuneate mossy fibers are gluatamatergic using double labeling techniques for PHA-L and glutamate at light and electron microscopic levels.

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There is evidence that cholineergic effects in the cochlear nucleus (CN) are mediated primarily by muscarinic acetylcholine receptors (MACH). We used [%]scopolamine to study MACH distributions in the rat CN by receptor autoradiography. Rats were anesthetized with pentobarbital 15 min thick were incubated with 10 nM [%]scopolamine at 25°C for 30 min in Tris with cold PBS-EDTA and a brief cold 0°C rinse. The sections were exposed to [%]sensitive film for 2 weeks, followed by exposure to NTB2 emulsion for 2 weeks. The sections binding were incubated with 4% albumin, and the radiomotope was estimated by measurements of optical density (using NIH Image 1.44, courtesy of Dr. E. Ties). The specificity of scopolamine binding was confirmed by blocking with 1 μM atropine. To determine which MACH subtypes are present in the binding, we included unlabeled subtype-preferential ligands in some preparations to block [%]scopolamine binding. Over 60 sections measured on isolated CN were included with [%]scopolamine and with unlabeled ligands, followed by scintillation counting. Our preliminary results suggest that scopolamine binding is highest in granular nucleus, followed in order by dorsal CN (DCN) deep and fusiform layers, DCN molecular layer, paracochlear CN, anteroventral CN, and auditory nerve root (which was similar to background). Ligands which preferentially block some MACH subtypes competed more effectively than others with scopolamine binding. (Supported by NIH grant DC 00172)


In this study, changes in cell size, GABA and glycine immunoreactivities, glycine receptor subunit expression and neurotransmitter release have been analyzed in auditory brain nuclei after unilateral and/or bilateral deafenation. Evaluations of these changes were performed by morphometrical and image analysis of the cells, of GABA and glycine immunocytochemistry, by in situ hybridization of glycine receptor subunits and the immunolocalization of microglia probes in brainstem auditory nuclei. Most of the neurons within the lateral superior olive (LSO) are able to shrink and then recover to their normal size after uni- or bilateral destruction. The morphological plasticity parallels changes in the density of GABA+ cell bodies in the LSO and in the central nucleus of the inferior colliculus. The fact that this plasticity of the auditory neurons is also observed in bilateral deafenation suggests that the central auditory system (CAS) of mammals has the property to recover even after total auditory deprivation. We also observe an ipsilateral decrease of glycine positive terminals in the medial nucleus of the trapezoid body (MNTB) and the dorsal mediolateral olivary nucleus in a simultaneous bilateral decrease following unilateral deafenation. Modifications of glycine immunoreactivity in LSO and MNTB appear to parallel some interesting changes in the expression of the different glycine receptor subunits. We are trying now to correlate these morphofunctional changes of the neurotransmitters with our results of microdialysis in the same experimental model. Even if the mechanisms behind these changes are still unknown, our results indicate that the CAS of mammals has a remarkable property of plasticity in response to partial or total auditory deprivation. Supported by MRT Grant # 88C0585 and NIDCD Grant # DC 00383.

400.15 LOCALIZATION OF GLYCINE RECEPTOR IN THE RAT COCHLEAR NUCLEUS AND SUPERIOR OLIVARY COMPLEX. K. Sato, H. Kuriyama, J. Dupont, J. Bousoum and R.A. Altschuler, Kresge Hearing Research Institute, The University of Michigan, Ann Arbor, MI 48109-0506.

Glycine is a major inhibitory transmitter that is especially prominent in the cochlear nucleus and superior olivary complex. The glycine receptor is comprised of α subunits, a β subunit and a 93 k anchoring protein (AP), also termed gephyrin. Different subunits can combine to form complexes with different physiological properties (intero- and extra-olivary). We have used radiolabeling and hybridization methods to localize glycine receptor subunits in the cochlear nucleus and superior olivary complex. This expression was then compared to immunolocalization of the 93 k AP. In the lateral superior olive (LSO) and medial nucleus of the trapezoid body (MNTB) the amount of expression of different subunits was compared to number of glycine immunoreactive terminals received as well pressure of 2%KI. Expression of α1 and α3 subunits were seen over principal cells of all major CN and SOC nuclei, while the expression of the α2 subunit was considerably lower. All cell types that showed expression of the catalytic alpha-ligand for the 93 k AP. While there was good correlation between the number of 93 k AP immunoreactivity and the amount of glycine immunoreactive afferent a cell received, there was no always a correlation between the number of silver grains over cells expressing receptor subunits and the glycine immunoreaction. LSO principal neurons receive many more glycinerich terminals than MNTB principal neurons. This is consistent with the increased expression of the α2 subunit of the β subunit.

Supported by NIDCD Grant # DC 00383.

400.16 SYNAPTIC ORGANIZATION WITHIN THE MEDIAL SUPERIOR OLIVE OF UNILATERALLY DAEERED GERBILS. E.A. Russell and D.R. Moore, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K.

Neurons of the medial superior olive (MSO) are thought to contribute to sound localization by signalizing the temporal correlation between excitatory input from the left and right antitiet (AVCN). Following the use of a non-reactive or new hybridization methods to localize neurons expressing glycine receptor subunits in the cochlear nucleus and superior olivary complex. This expression was then compared to immunolocalization of the 93 k AP. In the lateral superior olive (LSO) and medial nucleus of the trapezoid body (MNTB) the amount of expression of different subunits was compared to number of glycine immunoreactive terminals received as well pressure of 2%KI. Expression of α1 and α3 subunits were seen over principal cells of all major CN and SOC nuclei, while the expression of the α2 subunit was considerably lower. All cell types that showed expression of the catalytic α-ligand for the 93 k AP. While there was good correlation between the number of 93 k AP immunoreactivity and the amount of glycine immunoreactive afferent a cell received, there was no always a correlation between the number of silver grains over cells expressing receptor subunits and the glycine immunoreaction. LSO principal neurons receive many more glycinerich terminals than MNTB principal neurons. This is consistent with the increased expression of the α2 subunit of the β subunit. We are continuing this quantitative assessment and comparison.

Supported by NIDCD Grant # DC 00383.
400.17

The lateral nucleus of the trapezoid body (LNTB) is a prominent periventricular cell group that contributes a large feedback projection to the cochlear nucleus. This cell group is known to receive ascending activation via collaterals of small cochlear axons, although any detailed description of these terminals are sketchy. We have determined that FEP-19 antiseraum labels substantial numbers of fibers and terminals in the LNTB, including globular bushy cells, and ventral cochlear nucleus (VCN) axons. We studied the immunolabeled terminals using both light (LM) and electron microscopy (EM) to gain a clearer picture of globular bushy cell inputs to the LNTB.

Based on LM and EM descriptions, afferent GABAergic inputs to globular bushy cells in the LNTB can be identified. The first zone, located medially and ventrally and concentrated caudally, contains large FEP-19 IR puncta and fibers. The puncta in many cases form rings which define the perimeters of immunonegative cell bodies, and are typically on the order of 6-8μm. The second and larger zone is distinguished by having the highest density of immunonegative cell bodies. This zone contains large punctate profiles, although on average puncta are smaller than in the first zone and do not form pericellular rings. Pre-embedding EM immunocytochemistry reveals FEP-19 IR presynaptic boutons associated to unlabeled cell bodies and dendrites. Their internal morphology is consistent with their being excitatory terminals.

These results support the notion that LNTB neurons are rapidly and securely activated by bushy cells, and may exert rapid feedback effects on cochlear nucleus neurons.

Supported by NIH grant DC01387

400.19

Projections from the ventral cochlear nucleus (CN) to the opposite CN and to the inferior colliculus (IC) arise primarily from multipolar cells. In our first experiment, we injected different fluorouracil tracers into one CN and into each IC to determine whether individual cells project to more than one of these nuclei. Injection of the 3 tracers labeled the same cells in the uninjected CN; however, no individual cell in the ventral CN contained more than one tracer. Thus, commissural projections and collicular projections arise from different populations of cells. In a second experiment, we injected PHAL into one CN and examined the labeled axons in the other CN. In the ventral CN, labeled axons were observed to converge primarily onto cells whose somata were also aspicted to superficial and globular cell somas. Many labeled terminals were also found in the granule cell regions of the CN and in the deep layer and fusiform layer of the dorsal CN.

The commissural projections arise from cells that do not project to the IC. It appears that CN cells that do project directly to the IC, including a subset of multipolar cells in the ventral CN as well as cells in the dorsal CN, are themselves targets of the commissural projections. Other cell types, namely globular and spherical cells, are also targets of commissural projections. These cell types project to the IC indirectly, via relays in the superior olivary complex. The commissural projections thus provide an opportunity for binaural interactions at early stages in a variety of ascending auditory pathways.

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400.20
VENTRAL NUCLEUS OF THE TRAPEZOID BODY: DENDRITIC MORPHOLOGY BY INTRACELLULAR LABELING. N. Kuwabara* Dept. of Biol. Sci. and OUCOM, Ohio University, Athens, OH 45701.

The ventral nucleus of the trapezoid body (VNTB) is a periventricular cell group at the floor of the mammalian superior olivary complex (SOC). This nucleus is strategically positioned in the trapezoid body just below the medial nucleus of the trapezoid body (MNTB) and the medial superior olive (MSo). The VNTB contains a mixed population of elongate and multipolar cells and is known to receive extensive afferent inputs from the ventricular cochlear nucleus (VCN; Warr, ’72). Some cells of the VNTB are thought to contribute to the medial olivocochlear bundle system (Warr, ’75). The VNTB also has projections to both lateral and ventral collicular populations (Nordeen, ’83) and the cochlear nucleus bilaterally (Brown et al., ’88; Winter et al., ’89). However, little is known about the organization of the VNTB, its relation to the rest of the SOC, or the relationship between its afferent and efferent connections. We have begun our studies of the VNTB by examining its dendritic morphology as revealed by intracellular labeling in a gerbil brainstem slice preparation.

Intracellular labeling with Neurobiotin suggests that the main dendrites of most elongate VNTB cells are oriented dorsolaterally. Dorsally oriented dendrites often invaded and arborized within the MNTB. Some small dendritic branches were traced dorsolaterally into the columnar region of the MNTB. Ventral dendrites often extended across the entire width of the ventral trapezoid body. Previously, we have observed collateral axons from MNTB principal cells projecting to the VNTB (Kuwabara et al., ’89). The dendritic pattern of the VNTB suggests that this nucleus may sample ascending information in the neural plexuses of both the MNTB and the MSO as well as direct projections from the VCN. (Supported by DC01303, DC00383 and OUCOM).

401.1
SUPERIOR PAROVALVULAR NUCLEUS (SPON); CONNECTIVITY REVEALED BY TRANSPORT OF BIOTINYLATED DEXTRAN. E. Saltarini, D. R. Lough and A. S. Berrebi, Univ. of Salamanca Med. Sch., 37007-Spain, and Dept. of Otolaryngology, West Virginia Univ, Morgantown, WV 26506.

The SPON is a prominent periventricular nucleus located ventrally to the dorsal cochlear nucleus. In order to clarify its synaptic targets and sources of inputs, we injected biotinylated dextran (BD) into the inferior colliculus (IC) or SPON of rats. This tracer provides both retrograde and anterograde labeling of cell bodies and processes.

The projection from SPON to IC is extensive, primarily ipsilateral, and originates from large multipolar cells with flattened, parasagittally oriented dendrites, which are moderately situated SPON neurons project to ventromedial (high frequency) regions of the IC, and those situated laterally project to dorsolateral (low frequency) regions. Thus, the SPON projection to the IC is topographically organized.

After injections restricted to the SPON, an extremely dense fiber plexus, but very few cell bodies, are labeled in the IC. Numerous retrogradely labeled neurons were present within the ipsilateral region of the cochlear body (MNTB) and contralateral posteroventral cochlear nucleus (PVCoN).

The predominant neuronal type labeled in the PVCoN corresponds to octopus cells, although multipolar and scattered small stellate and bushy cells are also found.

These data suggest that SPON activity is modulated by the balance of its excitatory inputs from SPON and its inhibitory inputs from octopus cells originating in the MNTB. Since the SPON contains primarily inhibitory neurons, it appears that complex inhibitory/disinhibitory phenomena at the level of the IC may represent an important aspect of its functional role.

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401.2

Expression of the c-fos protein has been shown to be up-regulated in auditory brainstem neurons in response to tonal stimulation. In the present study we examined the expression of c-fos mRNA following 1 hr of free-field stimulation with a low-frequency, narrow-band noise (1.41-5.55 kHz) at intensities ranging from 80-120 dB SPL. At 80 dB elevated expression was found in subsets of neurons in the low frequency areas of the dorsal (DCN), posteroventral, and anteroventral cochlear nuclei, the medullary nucleus of the trapezoid body (MNTB), the ventral and dorsal nuclei of the lateral lemniscus (VLCN), and inferior colliculus (IC). The regions containing labeled neurons in these structures increased in size with 90 and 100 dB stimulation, but the signal began to decline at 110 dB. In some auditory nuclei, labeled in low-intensity areas was nearly absent, while low intensity stimulation may reflect damage to the low-frequency part of the cochlea. At 90 dB additional neuronal labeling was found in the highest frequency regions of some of these structures, particularly the DCN, MNTB, and IC. These areas of label also increased in intensity with increasing stimulus intensity, which may reflect the greater sensitivity of the high-frequency part of the cochlea to high intensities of all frequencies.

Alternatively, some central auditory neurons, i.e., those in high frequency regions of DCN, MNTB, and IC, may have more sensitive stimulus/transcription coupling mechanisms than others. At the highest stimulus intensities the middle frequency regions of the auditory nuclei became heavily labeled, presumably as the tail of the tuning sensitivity for these neurons was activated. c-fos mRNA expression appears to be related to a biophysical response driven by, but not necessarily synonymous with, neuronal activity. Its expression may be useful as an indicator of neuronal activity in many, though perhaps not all, neurons of the auditory brainstem.

Supported by the House Ear Institute, Research Service of the VA, and DC-00139.
401.3

EXPRESSION OF c-fos mRNA IN THE RAT AUDITORY BRAINSTEM FOLLOWING UNILATERAL OR BILATERAL COCHLEAR ABLATION.
L. Luu, R. R. Huang, and E. I.Abramoff, Int. House, University of Southern California, Los Angeles, CA 90007.

Transcription of the c-fos gene was examined from 30 min to 60 days after surgical ablation of one or both cochleas in adult rats using an in situ hybridization. After unilateral ablations, basal expression in the ipsilateral ventral cochlear nucleus (VCN) and contralateral inferior colliculus (IC) began to decline 1-2 hr post injury, was maximally depressed at 3-4 hr post injury, and remained suppressed through 60 days post injury. In the cochlear nucleus contralateral to the ablation there was a rapid (30 min.-1 hr post injury) increase in neuronal expression in the deep layers of the dorsal cochlear nucleus (DCN) and ventral cochlear nuclei which remained elevated through 60 days post injury. This suggests a release from inhibition normally driven by the contralateral ear. An intense glial expression in the acoustic and ventribuline nuclei of the ablated side, reflecting degeneration of the ablated ear, was first evident at 3 days post injury and gradually involved the nerve roots, octopus cell area, and spinal tract of the trigeminal. Elsewhere there were delayed increases in neuronal expression bilaterally in peristriate cortex, dorsal nuclei of the lateral lemniscus, paralemniscus, and commissural nuclei of the IC and in the ipsilateral ventral and intermediate nuclei of the lateral lemniscus and central nucleus of the IC. Peaks ranged from 2 hrs to 2 weeks and persisted for at least 60 days after ablation. The delayed onset of these responses suggests transynaptic effects and possibly reorganization of the central pathways in response to deafferentation in the adult. No neuronal expression was evident in any auditory cortex 4 hrs or more after bilateral ablations, suggesting that all observed expression was acoustically driven. A single exception was in the superficial layers of the DCN, where there was an initial rapid increase in expression in medium-sized neurons that reached a maximum at 2 hrs and disappeared by 1 day. This may reflect a release from acoustically driven inhibition of high spontaneous rate neurones with subsequent adaptation or delayed depolarization of higher order excitatory input.

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401.4

C-FOS EXPRESSION ELICITED BY SOUND STIMULATION IN THE AUDITORY NEURONS OF THE BIG BROWN BAT, EPTESICUS FUSCUS Y. Qian and P. H. S. Lem.
Division of Biological Sciences, University of Missouri-Columbia, MO 65211.

Because proto-oncogene c-fos can be expressed in neurons following sensory stimulation, c-fos immunocytochemistry has been used as a sensitive and selective method for identifying neurons that have undergone an activation pattern. We studied the immunohistochemical staining pattern of c-fos immediately following noise and tone burst stimulation of the auditory nuclei in the bat brain. Contralateral auditory nuclei were also studied with an emphasis on the dorsal cochlear nucleus (DCN). C-fos expression was studied immunohistochemically for the auditory cortex, the thalamus, and the superior colliculus.

401.5

STRUCTURE AND INNERVATION OF NEURONS IN THE VENTRAL NUCLEUS OF THE LATERAL LEMNISCUS THAT PROJECT TO THE INFERIOR COCCULUS. R. Berry, J. C. Beckers, and E. M. O'Leary, School of Medicine, University of Virginia, Charlottesville, VA 22908.

A multiplicity of pathways transmit auditory information from the cochlea to the inferior colliculus. While some of these are binaural, others may convey monaural information. One potentially monaural pathway which may lead to the inferior colliculus via the ventral nucleus of the lateral lemniscus (VNL). The VNL is a major source of input to the inferior colliculus, yet neither its inputs nor the morphology of its neurons have been extensively studied. Ascending input to the VNL was examined by injecting biotinylated dextran in the cochlear nuclei. Dextran-labeled axons that ran in the lateral lemniscus periodically gave off c-fos arborizations that entered the VNL. These horizontal arbor were roughly perpendicular to the lemniscus, and resembled the rango of c-fos arborization in the auditory thalamus. In a second experiment, neurons in the VNL that project to the inferior colliculus were labeled with retrograde transported fluorescent microspheres, and then intracellularly injected with a mixture of Lucifer Yellow and biotinylated Lucifer Yellow in slices of fixed tissue. The somata of stained neurons were spherical or elongated in shape. Differences were noted in the appearance of the ventral lemniscus, though most were more parallel to the lemniscus. Neuroticles with dendrites perpendicular to the lemniscus would likely receive input from one ear, or "wings" of fibers, from the cochlear nucleus, whereas those with dendrites parallel to the lemniscus may integrate inputs over a larger region. The relationships of dendritic orientation in the VNL to the input from the cochlear nucleus suggests that the VNL may play more than one role in auditory processing.

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401.7

MORPHOLOGY OF COMMISURAL AXONS IN THE INFERIOR COCCULUS OF THE GERBIL. Nina Z. Dorothenko and Neil B. Cape* Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

We made small injections of bicynoc into the central nucleus of the inferior colliculus and reconstructed the axons and terminals which arise from this injection and project into the contralateral inferior colliculus. All subdivisions of the inferior colliculus receive commissural connections; the patterns of branching are different in each one. Most commissural axons terminate in a manner typical of the central nucleus. Less often, labelled axons enter the central nucleus from its lateral dorsal aspect at right angles to the fibroblast laminar structure and travel in the ventromedial or lateral divisions of the central nucleus. The internal part of the external nucleus is innervated by axons which enter it by passing through the central nucleus, some of which give rise to branches. The terminal field of each axon is confined to a limited strip along the dorsal to ventral axis of the external nucleus. The superficial part of the external nucleus receives the sparsest intercellular connections. Most of the terminals there arise from branches of axons passing into the brachium. In the dorsal cortex, terminals of commissural axons are found most often in the middle of the deep layer. Most of them arise from branches of axons that enter the central nucleus or the brachium of the inferior colliculus.

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401.8

GABAergic PROJECTIONS FROM THE INFERIOR COCCULUS TO THE MEDIAL GENICULATE BODY IN THE CAT. S. Paylor*, R.Z. Stankewitz, B. L. Miller, M. D. Harley, and A. Winer. Dept. of Molecular and Cell Biology, Univ. of California, Berkeley, CA 94720.

The mammalian auditory system is unique among sensory modalities in that it has many brain stem projection areas that are GABAergic and probably inhibitory. These include ascending pathways (lateral lemniscus, trigeminal nerve [CN V], and accessory optic nerve [CN VI]), auditory nuclei (medial geniculate nuclei of both hemispheres), and basal ganglia structures. The mammalian auditory system is unique among sensory modalities in that it has many brain stem projection areas that are GABAergic and inhibitory. These include ascending pathways (lateral lemniscus, trigeminal nerve [CN V], and accessory optic nerve [CN VI]), auditory nuclei (medial geniculate nuclei of both hemispheres), and basal ganglia structures.

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401.10 CALCIUM-BINDING PROTEIN EXPRESSION DELINEATES SUBDIVISIONS OF RABBIT RAPHE, MIGRILICULE COMPLEX. N.T. McMillen, R.K. de Vries, P.R. Smogorz and S.E. Levin. Dept. of Anatomy, Univ. of Arizona College of Medicine, Tucson, AZ 85724

Previously cytoarchitectonically detailed studies of the mammalian medulla have recognized three major divisions: dorsal, central, and ventral. Additional additional parcelling of the medial medulla is warranted based on Golgi and intrinsic morphology. We analyzed the distribution of Ca**2+**-binding proteins (CaBP) in the rabbit medulla. By using specific monoclonal antibodies, we have characterized three major subdivisions of the rabbit medulla: dorsal, central, and ventral. These subdivisions were delineated using immunohistochemical methods and are distinct from other previously described subdivisions. The dorsal medulla contains a high density of CaBP, the central medulla contains a moderate density of CaBP, and the ventral medulla contains a low density of CaBP. These findings have implications for the functional and anatomical organization of the rabbit medulla.
401.15  
LOCAL PROJECTION PATTERNS IN THE RAT CORTEX DEMONSTRATED WITH BIOCYTIN  
D.F. Nowak* and F.B. Ciccarelli  
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Small intracortical injections of biocytin into the auditory cortex of the rat labeled the soma and processes of neurons exposed to the injected marker. In many cases, labeled processes were followed for considerable distances to targets in the ipsi- and contralateral cortices. In some cases, the injection was placed quite superficially in the auditory cortex layer VI, and in others, it was placed deeper into layers V/VI, producing different projection patterns. The laminar pattern of local circuit projections is dependent on the depth of the injection in the cortex. For example, an injection restricted to the superficial laminae in rostral auditory cortex (area 41), labeled neurons and processes around the injection site. Labeled processes were distributed most heavily in layers I, II/III, and V. A prominent fascicle of labeled processes descended into the subcortical white matter before turning abruptly dorsal, and then traveling to the corpus callosum. Bundles of labeled axons also passed horizontally through the cortex (not subcortical white matter), terminating in a columnar pattern in layers I, II/III, and V. The plexus of labeled processes in layer I was heaviest, followed by that in layer II/III, and then layer V. In contrast, when the injection involved the full thickness of the cortex, the local plexus processes terminated in a column extending through all layers. Long distance projections to ipsilateral visual cortices frequently took the most direct path to their targets, passing through the cortical laminae at oblique angles. Labeled axons were also observed in the corpus callosum. These axons entered the subcortical white matter directly beneath the injection, ran anteriorly, and passed to the opposite hemisphere at the lower half of the callosum. The results suggest that horizontal interactions among the auditory cortices have a unique laminar distribution that is dependent on the cortical lamina from which it arises. Supported by Veterans Administration  

401.17  
DEVELOPMENT OF AUDITORY CALLOSAL CONNECTIONS IN NORMAL AND HYPOPHYTALD RATS. R.A. Lucio*, J.R. Beracoa, P. Pacheco and P. Berbal  
Department of Histology and Neuroendocrinology, Univ. Alcance (Spain), and CIP, Univ. Auton. Talca, INE, Univ. Veracruzana and IB-UNAM (Mexico).  
Lack of thyroid hormones produces generalized brain damage, especially in the neocortex. Recent findings show that auditory callosal connections are severely damaged in adult hypothyroid (H) rats. In normal (C) rats, retrograde labelled neurons were found in cortical layers II-III, while in H rats, they were mostly distributed in layers IV to VI. To test for an abnormal elimination/establishment of transitory connections in H rats, the distribution of retrograde labelled callosal neurons in the auditory neocortex during development was compared with controls. For antithyroid treatment of H rats methimazole (Sigma) was orally administered from day 14 and a thyroidectomy performed on postnatal day (P) 6. At ages between P4 and P47, C and H rats were injected in the auditory cortex with HRP or WGA-HRP, and after 2 days, brain sections were processed with TMB. The data was analyzed with a "Neurograph" computer program. Until P35, the radial distribution of retrograde labelled neurons was similar in both groups. After P35, the distribution of labelled neurons was drastically reduced in C rats, reaching an adult-like distribution by P47. Interestingly, their number and radial distribution in H rats appears not to change from P35 levels, even during adulthood. These findings indicate that the transitory callosal connections normally eliminated, were retained in H rats.  

401.18  
AUDITORY CORtical PROJECTIONS TO THE PONTINE NUCLEI IN THE CAT. J.J. Fries*, R. Rigelens, M. Boccon, and J.A. Winer.  
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The pontomesencephalic nuclei are a major source of brain stem mossy fibers to the cerebellar cortex. Little is known about how the auditory cortex influences the output of premotor neurons in the brain stem. The sparse data available on corticopontine projections rely largely in studies which use autoradiographic silver degeneration methods. The goal of the present study was to demonstrate with more sensitive methods the pontine targets of corticofugal axons from four auditory cortical areas (A, AL, PAF, and TE). Each was injected with WGA-HRP, and frozen sections were developed for TMB. Axonal injections labeled axons in the dorsolateral, lateral, paramedian, and peduncular pontine nuclei. Injections in AL produced a lighter, but similar pattern of terminal labelling. With injections in PAF, most fibers terminated in the peduncular nucleus, while the projection to the dorsalateral and paramedian nucleus was scant. PAF was the only area that projected to the ventral pontine nucleus. TE was the origin of the heaviest projection to the pontine nuclei, and labeled fibers were observed in all of the dorsolateral and paramedian nuclei. TE differed from the other areas since it alone projected contralaterally, to the paramedian nucleus, and also in that it sent axons to the median pontine nucleus. These results demonstrate a convergence of auditory cortical afferents to the pontine nuclei, as well as other corticopontine projections that are specific to each auditory area. This suggests that multiple, descending corticofugal inputs to brain stem nuclei exist which may be analogous to parallel, ascending pathways to auditory cortex. Supported by grants PB 91-0736 and APC 93-0101 of the Spanish Government and by United States Public Health Service grant RO1 DC 03114-14.  

401.16  
The macaque monkey auditory thalamo-cortical system defined by calcium binding protein immunoreactivity. T. Hatakeyama, M. Mollair, M.E. Gage and E.G. Jones.  
E.G. Jones, Neural Systems Laboratory, Riken, Japan and University of California, Irvine.  
Immunoreactivity for calcium binding proteins is a useful marker to differentiates thalamocortical projections in the monkey. The present study was aimed at analyzing the pattern of parvalbumin and calbindin immunoreactivity in the auditory cortex and thalamus of the Japanese monkey (Macaca fascicularis) and to relate the parvalbumin and calbindin distributions. The auditory areas of the supratemporal plane have been divided into 4 almost concentric zones of decreasing parvalbumin immunoreactivity (Figure 1). Zone 1: intense staining corresponding to fields AI and RL; Zone 2: moderate to dense staining corresponding to anteromedial (A-m), lateral (L) and postero medial (P-m) parvicalcitol fields; Zone 3: weak to no staining at any zone to 2; Zone 4: weakest staining: anterior pole of the supratemporal plane. These zones appear to be related to the relative density of interneurons such as parvalbumin- and calbindin-immunoreactive neurons. Retrograde tracing studies demonstrated that zones 1-3 receive inputs from different subnuclei of the medial geniculate (MG) complex. A direct correlation was observed between the density of parvalbumin immunoreactivity in the cortex and that in MG nuclei. The anteromedial thalamic afferent to zone 1 (A + RL) receives projections from the ventral MG nucleus in which most cells are parvalbumin immunoreactive while 2 to 3 subnuclei from the anteriotal and posterodeal nuclei which show comparable differences in parvalbumin immunoreactive cell numbers. The present data demonstrate that parvalbumin immunoreactivity can differentiate parallel thalamocortical pathways of the monkey auditory system.  

402.2  
INVOLVEMENT OF THE INSULAR CORTEX IN NAUSEA: A C-FOS IMMUNOHISTOCHEMISTRY STUDY. P.A. Bryant*, B. Boutilier and I.S. McGregor  
Dept. Psychology, University of Sydney, NSW, 2006, Australia.  
Lithium chloride (LiCl) is frequently used to examine the nature of emesis in animals. Although rats do not have an emetic reflex, the reduced food intake and conditioned taste aversions (CTA), induced by LiCl in rats suggests that this substance induces nausea in this species. The insular cortex is a brain region containing both taste responsive and viscerosensory neurons and has been implicated in the acquisition and expression of taste-induced aversions. Lesions of the insular cortex interfere with LiCl-induced CTA learning in rats while epileptic foci in the human insula are associated with nausea and vomiting. Lesions of the insular cortex in rats also cause aphagia and in body weight loss, and electrical or chemical stimulation of the insular cortex alters metabolic processes and feeding; suggesting overall involvement of this area in ingestive function (McGregor & Abrens, Behav. Neurosci., 105, 870-883).  
The present study determined whether LiCl injection would activate insular neurons as indexed by c-fos immunohistochemistry. Rats were injected i.p. with either LiCl (Smk/g of 0.8M) or an equivalent volume of saline. The present study was performed under standard laboratory conditions for 4 days prior to LiCl. 4 hours after the injection, the rats were killed and the heads were fixed by perfusion with phosphate-buffered saline and 4%.formalin. The brains were removed and processed immunohistochemically for the protein Fos (a marker of neural activation). Rats injected with LiCl showed greater Fos expression in the insular cortex than rats treated with saline. This result suggests possible involvement of the insular cortex in nausea. Further research in our laboratory is aimed at understanding more fully this neural substrate and its role in nausea and CTA learning.

Although the precise nature of the relevant neural code is still the subject of some debate, the results of recent investigations by other laboratories appear to support the hypothesis that primary taste information is transmitted to the gustatory system in a binary code (on-off). The coding of more complex tastes (analogous in many ways to those associated with other sensory systems) transmit data related to the gustatory environment through the central nervous system. As evidence for this theory accumulates, it is important to determine the neural mechanisms for these information channels. In the present investigation, glass microelectrodes filled with 2.0% Neurobio (Vector Laboratories) were used to physiologically characterize and label individual gustatory neurons in the rostral nucleus of the solitary tract (NRT). We restricted our analysis to the responses elicited by NaCl, HCl, quinine and sucrose, we found a marginally significant relationship (p = 0.06) between best taste and the extent in the medio-lateral plane, with NaCl-best neurons more widespread than those that were most sensitive to quinine (α = 0.05). When we further restricted our analysis to those neurons that responded to all four of these tastes, we found that both NaCl-only and HCl-only neurons were more widespread in the medio-lateral plane than quinine-only cells. Furthermore, there was an indication that the quinine-only cells were more restricted in the dorso-ventral axis. The difference between these groups was clearly revealed when the area of influence was considered. Quinine-only cells were smaller in the coronal plane than the cells that responded only to NaCl, HCl or sucrose. These data support the hypothesis that the response properties of gustatory NST neurons are related to the morphology of these cells. Our results suggest that the relationship between responsiveness and dendritic architecture may be especially important. Support in part by DC01074.

GUSTATORY AND RELATED CHEMICAL SENSES


The rat reticular nucleus of the solitary tract (NST) contains second-order gustatory neurons, some of which project to higher brain centers via an obligatory synapse in the pontine parabrachial nucleus (pBN). The morphology of the projection neurons in the pBN has been described in rat (Laiwer and El Khoury, '83, Laiwer, '91) and hamster (Whitehead, '90), with some conflicting results. The first part of the current study re-examined the morphology, number and location of pBN projection neurons visualized by anterogradely labeling tracer Dil (Molecular Probes) unilaterally into the pBN in male Wistar rats (n=3) and examining the retrogradely labeled cells in the NST. On average, each injection produced 200 retrogradely labeled cells that could be visualized morphologically. Sixty-three percent of the labeled cells were located ipsilaterally to the injection. The labeled neurons were located throughout the NST, with the highest percentage (33%) found in the rostral NST subdivision. All neurons had either multipolar- or elongate-shaped somata with the majority (61%) being classified as elongates.

Evidence indicates that forebrain autonomic and limbic centers directly project to the NST (van der Kooy et al., '84). As a preliminary step to double-labeling studies investigating the neurotransmitter within these projections, we unilaterally injected DiI into the NST and examined the retrogradely labeled cell bodies found bilaterally within the amygdala, but none were found in the hypothalamus or other limbic structures. Cells in the amygdala were most abundant in medial, and particularly, basomedial structures with few cells located in central or lateral regions. Therefore, the basomedial amygdal nuclei may have direct effects on the processing of gustatory information within the NST.


IN VITRO ANALYSIS OF GLUTAMATE AGONIST EFFECTS ON NEURONS IN THE RAT OSTROBRAINSTEM. G.D. Shu and R.M. Bradley. Dept. Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109-1078.

Glutamate receptor agonists have been shown to have excitatory effects on neurons in the caudal nucleus of the solitary tract in rats (Andresen and Yang, Am. J. Physiol. 259:H1307, 1990). Using whole cell recording in brain slices of the rat mesencephalon, we have investigated the effects of a variety of glutamate receptor agonists, L-glutamic acid, and N-methyl-D-aspartate (NMDA) superfused with the NMDA antagonist, kainic acid, and NMDA receptors on the rostral gustatory area of the nucleus of the solitary tract (rNST). Recordings were made from neurons with stable resting membrane potentials and intact axonal connections. Superfused with glutamate receptor agonists, L-glutamic acid, and N-methyl-D-aspartate (NMDA) resulted in membrane depolarization usually accompanied by an increase in the number of AMPA receptors either increased their firing rate or became spontaneously active with the depolarizations. All neurons tested responded to NMDA and 83% responded to AMPA indicating that both NMDA and AMPA receptors on neurons. Neurons responded to NMDA and AMPA in a dose dependent manner. NMDA was effective over a concentration range of 10-100 µM and AMPA over a concentration range of 20-1000 µM. Reversal potentials for NMDA and AMPA were estimated from current-voltage relationships in control saline and in the presence of NMDA and AMPA at concentrations producing a maximal response. The NMDA reversal potential was 1.2 ± 0.2 mV (mean ± SE, n = 5) and the AMPA reversal potential was -4.9 ± 0.7 mV (n = 7). These results, together with our previous studies on synaptic potentials in NST neurons (Chem. Sens. 18:647, 1993), indicate that glutamate is an excitatory transmitter at the first central synapse in the taste pathway. (Supported by NIDCD grant DC00288 to R.M.B.)

We are interested in chemosensory transduction and synaptic mechanisms in the mammalian carotid body, an O2-sensing organ that regulates breathing (Hurtado et al., 2009). O2-sensory neurons are glomus or type 1 cells which transduce hypoxic stimuli by the closure of K+ channels, followed by depolarization and neurotransmitter release at synapses formed with petrosal sensory terminals. To investigate chemosensory signaling we developed co-cultures of dissociated (rat) petrosal neurons and glomus cell clusters. Monitoring of membrane potential in isolated co-cultured petrosal neurons, before and after perfusion of hypoxic (Po2=25-30 torr) solutions, revealed silent, as well as spontaneously-active neurons that showed no obvious response to hypoxia. A third, and most interesting category was juxtaposed to glomus cell clusters; 2 of 7 such neurons depolarized reversibly during hypoxia, from a typical resting potential of -55 to -70 mV, and this was accompanied by an increase in membrane conductance. These neurons are strong candidates for ones that develop de novo functional synapses with glomus cells, and will be useful for studying synaptic events during chemosensory signaling. Supported by MRC Canada.

402.11
CHARACTERISTICS OF ACTION POTENTIALS AND THEIR UNDERLYING OUTWARD CURRENTS IN MAMMALIAN TASTE RECEPTOR CELLS. Y. Yi, Y. Chen, and S. Xiao-Dong. Indiana University School of Medicine, Muncie, IN. 47306.

Many rat taste receptor cells conduct action potentials (APs). APs had a mean threshold of -35 mV (n=61 cells) and a spike height of 52mV above threshold in current clamp (hold = -80 mV). APs could be classified into two significantly different (p<0.001) groups - fast, with short half-time durations and large outward currents (mean=1.3 ms and 2.7nA), and slow, with long durations and small outward currents (mean=9.2 ms and 0.29nA). APs were conducted through TTX-sensitive sodium currents whereas the downstream by TEA-blockable outward currents. Voltage dependent analysis of outward currents separated transient and sustained components. The transient component was specifically blocked by 4AP (1mM). A calcium-dependent outward component was also revealed by modulating voltage and external calcium concentration. The fast recovery phase of the AP appears related to the sustained outward current whereas the after hyperpolarization (AHP) was blocked by 4AP suggesting a significant contribution of the transient component. Forskolin (FSK), which elevates cAMP, reversibly blocked the majority of the sustained current without influencing the transient. FSK greatly exaggerated the AHP without changing the spike height or duration. These data suggest that several components of the outward current contribute specifically to the gustatory AP and that the AP may be modulated by cyclic nucleotides. Supported by NIH DC00401.

402.12

Taste buds and the immediately adjacent epithelium are richly innervated both by specific gustatory fibers upon which taste receptor cells synapse and by free nerve endings. Classic anatomical studies have separated that many of the free nerve endings are innervated by capsaicin sensitive (i.e., substance P (SP) and CGRP) and that some of these enter the taste bud proper. Thus the designation of 'intragemmal' and 'perigemmal' probably does not correspond to the endo-epithelial role of the particular fibers. Further, a rich synapom- and synthaphylin-ir intragemminal nerve plexus is present and at least some of these fibers are post-synaptic to taste receptor cells. The present study on rats was undertaken to test whether the CRG/SP fibers and the synthaphylin fibers represent two largely independent and non-overlapping populations. In circumpalatine, a few scattered double-label fibers occur, while in the fungiform papillae, the two populations are mutually exclusive. Following perinasal treatment of rats with capsaicin (50 mg/kg i.p.), the SP/CGRP but not the synthaphylin-ir populations are abolished.

402.13

Focal chemical stimulation of the apical tips of intact taste buds elicits receptor potentials in receptor cells and postsynaptic responses in Merkel-like basal cells (Ewald & Roger, J. Neurophysiol. 67: 1316, 1992). There is evidence to indicate that in taste buds (Dickinson & Taylor and Roper, J. Comp. Neurol. 335: 606, 1993; Ewald & Roger, J. Neurosci. 13: 344, 1993) the synapses of receptor cells are postsynaptic responses in basal cells andafferrent nerve endings is unknown. In mammalian taste buds, acetylcholine (ACh) activates muscarinic receptors (Haas et al., 1988; 1990) and immunocytochemical evidence has shown the presence of choline acetyltransferase in taste buds (Kim & Roper, this volume). We have investigated the effects of locally applied ACh (10 μM to 1 mM) on cells in intact taste buds and on isolated taste cells from Necturus. Short pulses of ACh elicit hyperpolarizing responses in receptor cells (4.5 ± 1.3 mV; mean ± SE; N=5; ACh = 100 μM; Vs = -40 mV), accompanied by increases in input resistance (3 ± 12%). In intact taste buds these responses are enhanced by bath application of acetylcholinesterase inhibitor (10 μM neostigmine; 67 ± 6%; N=4). In isolated receptor cells, the responses are mimicked by oxotremorine (100 μM), a muscarinic agonist, blocked by atropine (30 μM), a muscarinic antagonist, and not mimicked by nicotine (100 μM). Responses are increased by hyperpolarization of the membrane potential. The zero intercept of this increase, in conjunction with an increased input resistance during the responses, suggests that ACh responses are primarily due to a transient decrease in resting Cl− conductance. We speculate that cholinergic responses may be part of an effector control circuit, or may play a role in lateral interactions between receptor cells. Support. NIH DC01238.

402.14

Glutamate (Glu) and GABA are found in nerve fibers that innervate Necturus taste buds (Jain & Roper, '91). Lu & Roper, '93). To investigate whether Glu/GABA are involved in synaptic transmission in taste buds, we studied their uptake and release in taste cells. Lingual epithelium was incubated with 3H-Glu (0.5, 5.0 μM) or 3H-GABA (0.6, 6.0 μM) in amphibian physiological saline (APS) for 15 min., rinsed with fresh APS, fixed with 2% glutaraldehyde, postfixed with 2% OsO4, embedded, and sectioned at 2.5 μm. Slides were coated with Kodak emulsion and exposed for 3-10 days. Background labeling for 3H-Glu was seen throughout lingual epithelium. However, silver grains preferentially accumulated over some taste cells. Next, epithelium was incubated with 3H-Glu and rinsed with APS containing 40 mM K+. K-reduction treated the number of heavily labeled taste cells, consistent with a depolarization-induced release of Glu. Rinsing tissues with 40 mM K+ but in the presence of 20 mM Mg2+ and 0.4 mM Ca2+ did not reduce the number of labeled taste cells. When lingual tissues were incubated with 3H-GABA, labeling was seen over cells lying outside of taste buds, including putative glial cells in the lamina propria. 3H-GABA uptake was not affected by rinsing tissues with 40 mM K+. We conclude that Necturus taste buds have uptake and Ca-dependent release mechanisms for Glu that may be related to synaptic actions. We did not find evidence for depolarization-dependent release mechanisms for GABA in taste buds.
402.15
IMMUNOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IN TASTE BUDS. Da-Jong Kim and Stephen D. Roger*. Dept. of Anatomy and Neurobiology, Colorado State University, Fort Collins CO 80523 and the Rocky Mountain Taste and Smell Center, Denver CO 80262.
Cholinergic synaptic mechanisms in taste buds were proposed several years ago on the finding that injecting cholinergic agonists and antagonists into the tongue modified taste responses (1). Additionally, acetylcholines- terase (ACHE) has been localized in taste buds from a number of species. However, ACHe can be associated with noncholinergic neurons, in force. The presence of ACHe is not a reliable indicator of cholinergic neurotransmission. In contrast, the localization of choline acetyltransferase (ChAT), the biosynthetic enzyme for acetylcholine, is considered direct evidence for cholinergic mechanisms in the nervous system. We have conducted immunocytochemical studies on lingual tissues from rats and mice to determine whether ChAT is localized to taste buds. Immunocytochemistry revealed ChAT in taste receptor cells of vallate papillae. Three to eight receptor cells per taste bud showed intense ChAT immunoreactivity in their cytoplasm. ChAT-immunopositive cells were elongate and their cell processes extended from the taste pore to the base of the taste bud. Immunostained cells were mainly located at the periphery of taste buds. On the basis of these data, as well as recent findings on the pharmacology of muscarinic agonists in taste buds (Hwang et al., PNAS 87: 7395, 1990; Ewald & Roger, this volume, 1994), we postulate that acetylcholine functions as a neurotransmitter in vertebrate taste buds.
Supported by NIH DC00374 and DC00244.

402.16
Chorda tympani (CT) transection causes anterior tongue taste bud degeneration and severely disrupts the rat's ability to discriminate NaCl from KCl. We recently demonstrated that rats trained presurgically and tested starting 49 days post-surgically (CTX/49d) could discriminate the two salts, but those tested 8 days post-surgically (CTX/8d) could not. The rats tested starting 28 days after bilateral CTX (n=7) displayed variability in salt discrimination performance, ranging from normal to severely impaired (overlap score range: 0.7-77.9). A third group (n=6) tested 49 days after CTX displayed competent discrimination. When the data from the present groups were combined with the data from the control, CTX/49d, and CTX/8d groups previously reported, the overlap scores correlated (r=0.88) with the percentage of fungiform papillae containing a TP (p<0.001, n=7). Although some rats with a small number of anterior tongue TPs were partially competent, overall it appears that there is a strong relationship between the extent of taste bud regeneration and performance in the salt discrimination task. Supported by PHS grant DC-01638.

403.1
SIMPLE AND COMPLEX RELATIONS OF MOTOR CORTICAL ACTIVITY TO GRIP FORCES IN THE ALIEN MONKEY. H.J. Qi, E.J. Huster, I. Ali, M.C. Hepp-Reymond*. Brain Research Institute, 8005 Zurich, Switzerland.
Neuronal correlates of force have been repeatedly shown in the primary motor cortex (M1). We wondered whether the neuronal activity in two ventral premotor regions (PMv) and M1 may code other features than just grip force.
We have analysed 242 finger-related cells, recorded from the 3 regions in 2 monkeys trained to produce isometric force in a step-tracking task. The trials varied in number of steps, their range and direction, each alternating specified by its own colour cue. The findings are: 1. More than half of the neurons displayed positive or negative correlation between cell activity and force. In 70% of these significant cells, similar direction discharge patterns were found under all test conditions. The rest, and a large proportion of non-significant cells, had more complex firing patterns. Most of these neurons (df cells) changed their force correlation at the highest level of linearity only within a narrow force range. With high forces a few df cells also altered their phasic activity component during the force ramps. The majority of the neurons with complex force relation (df cells) clearly changed their discharge patterns during the last hold period, regardless of the type of trial. 2. The distribution of the 3 cell populations and their mean force sensitivity was comparable in the 3 explored regions. 2. Most neurons with significant correlation coefficient responded to activation of deep (muscle and joint) receptors. The proportion of cutaneously activated cells was higher in the df population. 4. Some PMv neurons with receptive fields on face and hand also showed significant covariation with force.
In conclusion, these findings suggest that most M1 and PMv neurons code force in simple manner, and that only a minority has activity related either to force within a narrow range or to other parameters or task features.

403.2
Two macaque monkeys were trained to perform a two-dimensional, delayed step-tracking task in which they used the right upper extremity to operate a joystick that moved a cursor on a video display screen. Single cell recordings were obtained from the shoulder and elbow regions of the primary motor cortex. The presentation of a fixation target on the center of the monitor signaled the beginning of a trial. Once the monkey moved the cursor into the fixation point, four peripheral targets were presented. One of the target subsequent movements of the joystick was used to direct the monkey to capture the target after a delay upon the dimming of the fixation point. Spatial mapping between the cursor and joystick movement was offset by either 0° or 90°. The head trajectory was thus suggestive of the direction of the direction of displacement (from center) of a given target. The spacing of the targets was such that the set of movements towards all four targets was equivalent in the two conditions, allowing determination of whether neurons performed different responses were detected. Tuning curves of the first type of cells were invariant with respect to hand trajectory. The second type showed different responses that were invariant with respect to different hand trajectories. These results indicate that directional tuning of motor cortex neurons is not always representative of limb trajectory as neurons of the second type appeared to reflect the intended direction of motion towards a given target rather than that of the limb.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
CELL ACTIVITY IN MONKEY MOTOR CORTEX IS ALTERED BY CHANGES IN POSTURE FOR MOVEMENTS WITH IDENTICAL HAND-TRAJECTORIES S. H. Scott* and J. P. Kalaska, Dép. de Physiologie, Univ. de Montréal, Montréal, Québec, CANADA, H3C 3J7.

The debate continues as to whether neuronal activity in the monkey motor cortex during reaching movements is better related to the extrinsic (i.e. hand trajectory) or intrinsic (e.g. muscle activity) attributes of motor task. We have trained a monkey to move a pendulum-like handle to visual targets using two different arm postures, but with identical hand paths. In the first posture (horizontal), the monkey showed to perform the task in its preferred arm orientation (largely in the sagittal plane). In the second posture (vertical), the monkey had to perform the task approximately 90 degrees in grasp and move the handle. This altered arm posture changed the mechanical properties of the muscles (length and moment arm about the joint) and changed the EMG activity patterns of muscles that span the shoulder and elbow joints. We recorded the activity of a large sample of neurons, joint-pen-event histograms (Aertsen et al., 1989) were then calculated on 205 pairs of simultaneously recorded neurons. The activity of 39 pairs of neurons (15%) was significantly synchronized during periods of time lasting 50 to 300 ms. Such periods of synchronization occurred just after the preparatory signal, at the end of the PP, during RT and/or during movement time. For 14 pairs, the neurons fired with a synchrony of less than 1 ms, whereas in the remaining 25 pairs, larger peaks of cross-correlograms were observed. Synchronized neurons were recorded with a mean horizontal distance of 377 μm (160 to 1980 μm) and a mean vertical distance of 464 μm (0 to 1810 μm). This study provides further evidence for dynamic changes in the functional connectivity within neuronal populations involved in the processing of sensorimotor information. Detailed analysis of cross-correlation data will be used in an attempt to decipher the functional organization of the recording process in the central base and to investigate how cognitive functions are implemented in the microstructure of the cerebellum.

403.6 PRIMATE PREFRONTAL CORTEX PLAYS A SIGNIFICANT ROLE IN PERFORMING SEQUENTIAL BEHAVIOR WITH AN IMPOSED DELAY. S. Panagopoulos*, M. T. and C. Grant, Grad. School of Human and Environmental Sciences, Kyoto Univ., Kyoto 606-01, and Prime Res. Inst., Kyoto Univ., 630-8620, Japan.

To investigate the roles of the prefrontal cortex in generating sequential behavior, single neuron activity was examined in the prelpirpical region while 2 monkeys performed a sequential task involving two of three targets: press the button to reach to one target as a control. In addition, to examine whether prefrontal neurons retain multiple target positions simultaneously, a 3 delay period was imposed between the target presentation and movement time. A total of 234 single neuron activities were analyzed. Among them, 138 exhibited task-related activity in at least one out of the three phases: trial, 87; 3-5, 108 exhibited cue, delay, and response-related activity, respectively. A large proportion of these activities exhibited complex and context-dependent characteristics. For example, 50% of the delay-related activity was observed only when the targets were presented in a particular order during the cue period (pair- and sequence-dependent), and 18% of this activity was observed only when a specific target was presented in either the first or second position (position- and sequence-dependent). For the remaining neurons, targets related activity under simple conditions. For example, 8% of the delay-related activity was observed when the target was presented in a particular position, regardless of its order. A similar variety of response characteristics, from context-dependent to simple, was observed in response-related activity.

These results indicate that the prefrontal cortex plays an important role in executing sequential behavior. The prefrontal cortex may participate in complex sequential behavior by retaining target positions through a variety of activities, each of which can retain either simple information (e.g., a single position) or multiple and complex information (e.g., multiple positions and a particular order).

403.8 MOTOR CORTEX AND INTERCEPTION OF MOVING TARGETS: SINGLE CELL ANALYSIS. N. Lindstrom*, W. Kiste, P. Dansonville and A. P. Georgopoulos, Brain Science Center, VAMC, Minneapolis, MN.

Two monkeys were trained to use a 2D articulating manipulandum to intercept moving targets on a computer screen. In random order, 9 targets either accelerated, decelerated or traveled at a constant velocity. For each motion condition targets were traveled for one of three target presentation times: 0.3, 1 or 2 s. Each target appeared randomly in either the right or left lower corner of the screen, then traveled along a 45° path until they crossed the vertical meridian. The monkeys were required to intercept the moving target as it crossed the vertical meridian, making an upward movement (12 cm) from an initial hold position. An interception was considered successful when the moving target entered, within 130 ms of the target, a 1 cm radius positional window centered on the point of the target after it crossed the vertical meridian. The spike activity of 411 cells were recorded in the arm area of the motor cortex in one monkey during task performance. We found that the activity of various populations of cells was modulated during the interception task. In most cases the change in cell activity was similar to that observed during upward movements toward a stationary target, in another task, but in other cases changes in cell activity differed. For example, cell activity during the last 200 ms of the reaction time was frequency modulated in relation to the temporal characteristics of the ensuing movement: for example, activity in 8% of cells differed significantly (P<0.05, ANOVA) among trials with different target velocities, irrespective of the kind of target motion (accelerating, decelerating or constant speed), whereas in 6% of cells it differed according to the kind of motion above, irrespective of the speed itself, and in 11% of cells both speed and kind of target motion had a significant effect. These results indicate that the motor cortex is involved with processing of the temporal characteristics of the interception movement. (Supported by NSF grant PSMH41885)
MOTOR CORTEX AND 3D ISOMETRIC FORCE: STATIC RELATIONS. J. Boline*, J. Ashe, M. Taira and A.P. Georgopoulos. Sciences Center, Veterans Affairs Medical Center, and Dept. of Physiology, Univ. of Minn. Med. School, Minneapolis, MN

The relations between the steady-state activity of cells in monkey motor cortex and the direction and magnitude of isometric force were determined using a 3D isometric manipulandum (Massey et al., J. Neurosci. Methods 26:123-127, 1988) and a visually instructed task. Five repetitions of 24 constant force levels were employed in a randomized counterbalancing design where each block consisted of 3 force magnitudes (100, 150 and 200 total gm-force) exerted in each of 8 XYZ directions (every 45 deg); force exerted in the Z dimension was monitored but not controlled. Data from electromyographic (EMG) activity of 7 (averaged) single units for each of 3 monkeys were analyzed using a repeated measures analysis of variance and a multiple linear regression of EMG and cell activity versus the X, Y, Z components of force. The magnitude of force had a significant effect (P<0.05) in 75% of muscles and 31% of cells studied, whereas the direction of force had a significant effect in all muscles and 70% of cells; significant magnitude x direction interactions were observed in 62.5% and 23% of muscles and cells, respectively. The multiple regression was significant in all muscles and 76% of cells. However, the regression was different in each muscle and/or cell. This difference in regression coefficients may reflect differences in the functional role of the neurons that were sampled. The results suggest that the neuronal discharge in motor cortex is related to the overall magnitude of the force exerted, but not to the direction of force exerted. This knowledge can perhaps be used to improve control of neuromuscular activities in patients with motor disabilities.


The hypothesis was examined that dynamic processes, effecting change in force, and static processes, involved in the maintenance of a constant force, are reflected separately through the patterns of neuronal activity of cells in monkey motor cortex. For that purpose cell activity was recorded while monkeys performed 3 visually instructed tasks using a 3D isometric manipulandum (Massey et al., J. Neurosci. Methods 26:123-127, 1988). The tasks involved the production of (a) purely dynamic isometric force (Task 1, n=430 cells); (b) step-and-pace forces that were maintained for 0.5 s (Task 2, three step forces; n=91); and (c) dynamic force pulses in the presence of a constant force bias (Task 3, n=331). Results from tasks 1 and 3 showed that cells associated with the force pulses were similar across different force biases. Results from tasks 1 and 2 showed that the time course of cell activity in task 2 resembled initially (during the reaction time) the pattern observed in the dynamic pulse task, and then changed to the pattern appropriate for the ensuing static force step. The replacement of the dynamic by the static pattern occurred approximately 150 ms before the attainment of the static force level. Moreover, the dynamic and static patterns were frequently directionally incongruent. (Supported by NIH and VA).


Spikes triggered averaging of rectified EMG activity has proven to be an effective means of identifying motor cortex cells with a functional linkage to motoneurons and motoneuron pool. Brain Res., 247:311 (1982). Previous studies of forearm muscles have shown that many CM cells not only facilitate multiple agonist muscles of the forearm but also suppress the antagonist muscles (Kawamura, J. Neurophysiol. 53: 85-96, 1985). This reciprocal output pattern constitutes a functionally meaningful synergy raising the possibility that more complex functional synergies may also be represented in the synergistic outputs of single CM cells. The purpose of this study was to investigate 1) the possibility that during an arm reaching movement, individual CM cells may facilitate muscles at different joints; and 2) the extent to which the facilitated muscles constitute a functional synergy whose action would produce a specific phase of the movement. The spike discharges of single cells from CM were recorded in the precentral gyrus of the monkey and one averaged sample of rectified EMG activity from 22 muscles of the shoulder, elbow, wrist and hand while the monkey performed an automated reaching task to retrieve a pellet from a fixed location. The monkeys were trained to reach in the direction of the stimulus light through the ipsilateral (flexion) or contralateral (extension) (extension) as compared to away from it (extension). Premovement activity onset for both CM and NS cells preceded movement onset by about 150 ms while the earliest EMG activity recorded from both CM and NS muscles was about 90 ms. The CM cell related neurons were significantly more responsive to vibration during After trials. These observations suggest that monkeys are more attentive and that some CM and NS neurons are more responsive to peripheral and central inputs during unpredictable movements. These inputs may be related to the 90 ms latency period between the occurrence of the Premovement activity and the sensory input. (Supported by NS 17413).
SYNCHRONIZATION OF PRIMATE SENSORIMOTOR CORTEX NEURONS DURING 20-40 HZ FIELD POTENTIAL OSCILLATIONS. V.N. Sushchov* and E.E. Foster. Regional Primate Research Center, Univ. of Washington, Seattle, WA 98195.

In monkeys performing exploratory and manipulatory free limb movements, local field potentials (LFPs) in sensorimotor cortices contained 20-40 Hz oscillations in the 20-40 Hz range. Previous findings that LFP oscillations occurred synchronously in different sites have been extended to the associated changes in single/multi unit activity. Cycle-triggered histograms (CCHs) of unit activity and cycles of LFP oscillations revealed modulation in two bands (176/568) of recorded units. Peak spike activity occurred 7.5 ms prior to the peak LFP negativity, or -5 ± 1.3 ms. Average power and coherence of oscillation amplitude (amplitude of oscillatory component of CTH as percent of baseline) was 45 ± 15%. Cross-correlation histograms (CCHs) between pairs of ipsilaterally recorded units were compared. When all spikes were used, moderate oscillation coherence was reflected by significant features. However, CCHs compiled selectively from up to 12 pairs of spikes were more frequent, with the peak (-5 ± 1.3 ms) corresponding to the peak power of LFP oscillations. These results are consistent with the possibility that oscillations are transiently synchronized by neural activity, not being limited to stable projections of the same unit. Thus, oscillatory synchronization does not appear to be a general property of neuronal interactions. During oscillatory episodes of the first rank firing rate, some units tend to be restricted to values corresponding to the average rate plus or minus the standard deviation.


The objectives were to determine whether primary motor cortical areas, evoking contractions in synergistic antagonistic muscles, received inputs from common or separate sources. Intra-cortical microstimulation (ICMS) was used at low-threshold areas capable of evoking limb movements. From this map, sites capable of evoking ankle flexion, ankle extension, and lateral flexion were selected for injection of fluorescent tracers. The procedures were (1) injection of fluorescent tracers into the corticospinal motor output sites, (2) label retrograde transport for 3-5 days, (3) section the brain, and (4) map distributions of differently-labeled neurons. Computer controlled injections yielded 0.3-1.1 mm columns of densely filled tracer which was inactivated in the extracellular cortical sites. There was no overlap in the distribution of neurons labeled with different tracers when using small injections but this increased when using larger injections. Conversely, ventral thalamic areas did not show increased overlap with larger injections despite the presence of overlap of intralaminar thalamic areas with all injections. This study suggests that some cortical and thalamic areas provide a single command to the motor cortex for the control of coordinated muscle actions while other cortical and thalamic areas separately control individual muscle actions. [Supported by Canadian MRC]


The mechanisms by which neurones perform spatial and temporal integration are dependent on the numbers, types, and distribution of synapses they form with their afferents. The data presented, indeed indicate that these synaptic mechanisms, the distribution of synapses with identified classes of cortical projection neurones was examined using ultrastructural methods. Pyramidal cells in the cat motor cortex were the somatic overlap or to the motor cortex labeled by the retrograde transport of HRP. The somata of corticocortical and cortico-spinal neurones were identified using serial-section electron microscopy. The profiles of these somata and the synapses formed with each of these profiles were reconstructed from each thin section with the aid of a computer-aided morphometric system. The profiles of the synapses on the somata of pyramidal neurones were of the symmetrical, presumably inhibitory type. These synapses were not distributed at random in the neocortex, but were clustered at several zones, particularly in the vicinity of the axon hillock. The nonuniformity in the distribution of axosomatic synapses indicates that analyses of synaptic inputs of somatocortical neurones must be made in terms of the complete somatic surface area, and that studies in which small portions of these structures are randomly sampled may be misleading. The number, density and spatial distribution of synaptic inputs are identical on the soma of neurones of different corticocortical cells. In contrast, different corticospinal cells formed varying densities of inhibitory synapses. These findings indicate that different classes of cortical neurones receive unique patterns of inhibitory inputs and support the hypothesis that the synaptic organization of the cerebral cortex is highly specific. [Supported by NIH grants #NS31078 and #NS31078].

INDEPENDENT ANATOMICAL CIRCUITS FOR REACHING AND GRASPING LINKING INFERIOR PARietAL SULCUS AND INFERIOR AREA 6 IN MACAQUE MONKEY. M. Motteri, G. Luppino, A. Murru, and H. Sakata.* [SPON: European Brain and Behavior Society] Istituto di Fisiologia Umana Università di Parma Via Gramsci 14-14510 Parma (Italy); J Dept. of Physiology, Nihon University, Gyoda, Saitama, Tokyo 175, Japan.

Recent physiological evidence showed that in the intraparietal sulcus (IPS) and inferior area 6 there are areas that have functionally similar functions but are functionally separated. Intraparietal cortex (IPC), which lies in the lateral bank of IPS, and F5, located in the rostral part of inferior area 6, are both involved in planning and carrying out both grasping and manipulation movements. Ventral intraparietal area (VIP), which lies in the fundus of IPS and F4, located in the caudal part of inferior area 6, are both involved in coding the proprioceptive signals for possible reaching movements.

In order to see if these functionally similar parietal and frontal areas have direct and selective anatomical connections, we monitored the connections of neurons in different corticocortical areas (VA, VP, and IP) in F5, F3, and LIP, projecting to and from the macaque monkeys. After injections of F4 the labeling was found only in the ipsilateral thalamus and ventral premotor cortex. The injections in F5 VIP was spared and the labeling was almost completely confined to AIP. The selective unanatomical linkage between AIP and F5 was fully confirmed by tracers injections in AIP. The results also showed that different regions were confined to the sector of F5 located in the arcuate sulcus. A caudal injection in the lateral bank of IPS (possibly LIP) produced labeling in prefrontal cortex. Inferior area 6 was devoid of any labeling. The present data indicate that visuomotor integration for reaching and grasping are processed along independent pathways. [Supported by a Grant from Human Frontier Science Program].

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
1) AN ELECTROPHYSIOLOGICAL STUDY OF THE CORTICORRETICULAR PATHWAY IN THE INTACT, AWAKE CAT. K. Bouchar and T. Drew. Dept of Physiology, University of Montreal, Canada.

As part of a study to determine the functional organization of the corticoreticular pathway in the cat, we examined the projection patterns of 459 neurones within layer V of the pericruciate cortex (areas 4 and 6) of each cat, each of which was implanted, bilaterally, with 6 arrays (each of 6 wires) of microelectrodes. (The study was supported in part by NIH Grant NS 25169 (P5-P11; L0.5-1.1.5). In addition, microwire electrodes were inserted into the pyramidal tract (PT) at a caudal level (P14.5), close to the decussation. We used a similar protocol in the monkey (A. Cabana et al., 1985).) The corticofugal neurones projected only as far as the MRF (corticoreticular neurones: CRNs); 17/125 (13%) neurones were activated both from the MRF and from the PT (CRN/PTNs); and 24/129 (19%) were identified only from the PT (PTNs). In area 4, in contrast, only 62/330 (19%) cells CRNs; 88/330 (27%) were CRNs/PTNs; and 154/330 (47%) were PTNs. Furthermore, 20/35 (58%) of the neurones in Area 6 and 45/159 (30%) of the neurones in Area 4 that projected to the MRF were activated from more than one injection site. (The study was supported in part by NIH Grant NS 25169.) In both areas 4 and 6, 7/125 (6%) of the neurones were classified as short, whereas 4/129 (3%) of the neurones were classified as long. The results suggest that the major projection to the MRF from Area 6 is from slowly conducting CRNs, whereas the projection from Area 4 is both from slowly conducting CRNs and more rapidly conducting CRN/PTNs. (Supported by the FCAR and the MRC.)


To quantitatively examine the zones of termination of the corticofugal pathway to the lower brainstem, Phasengus vulgaris (PHA) was injected into either the forelimb regions of motor cortex (Area 4), or into the more dorsal (N=2) or more ventral (N=1) regions of the premotor cortex (Area 6). A light microscope, equipped with photomultiplier counters and a computer system, was used to measure the X and Y coordinates of the PHA-labelled cells found in every fourth transverse section of the ponto-medullary region (section thickness=50um). In all cases, the greatest density of terminals was found in the ipsilateral pontine gray (PG). Jjections into dorsal Area 6 resulted in a widespread distribution of terminals, while the labelling from the dorsal Area 6 injections was more restricted to the medio-ventral regions. Injections in Area 4 resulted in a similar distribution of labelled terminals, but with a weaker density of labelling than those produced by Area 6 injections. (Supported by the Canadian MRC and the FRSQ.)
ATTENTION TO A VISUAL STIMULUS ENHANCES NEURONAL RESPONSES IN MONKEY PREFRONTAL CORTEX. Y. Kodaka, A. Mikami and K. Kubota. Dept. of Behavioral and Brain Sci., Primate Res. Inst., Kyoto Univ., Inuyama 444 Japan
To investigate how selective attention modulates neuronal activities in the prefrontal cortex (PFC), we recorded neuronal responses to the identical visual target in non-attentive and attentive conditions in the monkey PFC. In a visual fixation task (VFT), while the monkey gazed at a fixation point, a visual stimulus, to which no behavioral meaning was assigned, was presented extraretinally in its visual receptive field. In a visual peripheral cueing task (VCT), the monkey released the lever within 800ms after an identical stimulus was dimmed. Activities of 48 neurons in the prefrontal and periprefrontal areas were compared under these two conditions. In 38% of the neurons, the magnitudes of the responses in the VFT were significantly larger than those in the VFT. For each neuron, the visual response tended to be greater toward the center of the RF. In the remaining 62% of the neurons, the magnitudes of the visual responses in the VFT and VCT were similar. Neither eye movement nor lever release occurred when the visual stimulus appeared. Therefore, these enhancements were not causally related to either eye movement or lever release. These results suggest that enhanced responses due to focused attention to the visual stimulus in the PFC may facilitate the selection of a behaviorally significant stimulus within the RF.

2-DEOXY GLUCOSE (2DG) UPTAKE IN THE MEDIAL WALL MOTORS AREAS OF BEHAVING MONKEYS. N. Prasad and P.L. Deer3. VA Medical Center and Dept. of Neurosurgery and Physiology, SUNY Health Science Center, Syracuse, NY 13210.

The medial wall of the hemisphere contains multiple motor areas. They are the supplementary motor area (SMA), the Pre-SMA and three cingulate motor areas (CMAd, CMAc and CMAp) (Dum and Strick, 1991). We have begun to investigate the relative involvement of these motor areas in different aspects of motor function using the 2DG method. Two monkeys were trained to perform different sequences of reaching movements for a juice reward (REM task). Two other monkeys were trained on a Control task to sit in the primate chair and lick juice rewards delivered at times intervals comparable to that of the reaching monkeys. Two areas of the medial wall showed significant activation in the REM animals and not in the Controls. For the REM task, 2DG labeling was particularly prominent in the CMAp and the Pre-SMA. Focal activation was found in the region of the CMAp that projects to cervical segments of the spinal cord and arm areas of the primary motor cortex. Initial activation was present on the upper median (primary) gyrus, particularly in the territory of the Pre-SMA. Labeling in other motor areas on the medial wall was either absent or less significant. These observations suggest that, of all the motor areas on the medial wall, the CMAp and the Pre-SMA are preferentially involved in the internal guidance of sequential voluntary arm movements.

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CINGULATE CORTEX SUBDIVISIONS IN THE HUMAN AND MONKEY REVEALED BY STAINING FOR PARVALBUMIN. A. Solotkin1,2, R.J. Morecraft1 and G.W. Van Hoesen3. Departments of Anatomy and Neurology, University of Iowa, Iowa City, IA 52242.1 Department of Anatomy and Structural Biology, University of South Dakota, Vermillion, SD 57069.

The cingulate has been implicated in a wide range of behaviors including motivation, attention, mechanisms relating to pain and motor control. Not unexpectedly, its structure is complex differing both in anterior-posterior and medial-lateral dimensions. We have studied the topography of cingulate subdivisions in humans (N=15) and in the non-human primates (N=9) using immunohistochemical techniques for the calcium-binding protein parvalbumin (SM-32, Stemmer). Krieg and Sarkissian's cytoarchitectural parcellation of 24a, 2b and 23a, b was used as a guide. Our observations indicated that parvalbumin immunostaining is an excellent method to delimit different subfields in the cingulate cortex in both humans and monkey. Since this calcium-binding protein coexists with a subpopulation of GABAergic neurons, its discrete distribution suggests probable functional differences between cingulate subfields. Supported by NS 14944 and PO NS 16832.

THE HUMAN FRONTAL MESIAL CORTEX: A COMPARISON BETWEEN CYTOARCHITECTONIC, RECEPTOR AUTORADIOGRAPHIC, AND FUNCTIONAL ACTIVATION. K. Zilles1, G. Schlag2, G. Geyer1, T. Schormann1, R. Seitz2, H. Steinmetz2 and H-J. Freund2. Brain Research Institute1 and Department of Neurology2, University of Düsseldorf, 40001 Düsseldorf, Germany.

Four supplementary motor areas (SMA proper, pre-SMA, caudal and rostral cingulate motor areas) are described in the literature. These areas were demonstrated by PET studies in the human frontal subcortical cortex. The aim of our study is the identification of cytoarchitectonic areas representing these functionally defined regions and the analysis of the regional distribution of tracer receptors by quantitative in vitro autoradiography in these areas. Several architectonically and receptor autoradiographically definable areas are found in the mesial frontal lobe and in the lower bank of the cingulate sulcus. By comparison with PET studies, the caudal-mesial part of Area 6 (mesial Area 6ax) is probably identical with SMA proper, the rostral-mesial part of Area 6 (mesial Area 6d) with pre-SMA, and the caudal or rostral parts of the cingulate areas showing large pyramidal cells with the caudal or rostral cingulate motor areas, respectively. Our study shows for the first time that within the human mesial frontal cortex multiple, probably supplementary motor areas are definable by architectonic characteristics and by receptor autoradiography. New cytoarchitectonic landmarks are defined, which allow the approximate location of these areas in coregistered PET/MRI images. Supported by the DFG (GBR 194, HP6 and HC3).

NEURONAL ACTIVITY IN THE SUPPLEMENTARY AND PRE-SUPPLEMENTARY MOTOR AREA IN RELATION TO SEQUENTIAL PERFORMANCE OF MULTIPLE MOVEMENTS. K. Shima and J. Tanji. Dept. of Physiology, Tohoku University School of Medicine, Sendai, 980 Japan.

The purpose of the present study was to examine how neuronal activity in the supplementary motor area (SMA) and presupplementary motor area (pre-SMA) is involved in performance of multiple movements. We trained monkeys (Macaca fascicularis) to perform three different movements (push, pull or turn a manipulandum) sequentially, separated by brief waiting periods. Each movement was triggered by a tone signal. After completion of individual movements, the manipulandum was returned to the neutral position within which the animal had to hold it and wait for next movement. In one condition, a visual signal (LED of three different colors) indicated what movement to be performed. In the other condition, colors of the three movements were randomly presented. Our results indicated that the sequential performance of the three movements was remembered. By contrast, a majority of pre-SMA neurons exhibited greater activity when the movement was instructed by visual signals. In both areas, there was an abundance of neuronal activity that was related to the temporal order of the three different movements, rather than related to next movement to be performed. Such neuronal activity, useful for programming the sequence of multiple movements, was not observed in the primary motor cortex. (Supported by Japanese Ministry of Education, Culture & Science 06NP0101.)

Cingulate Projections to the Basal Ganglia, Red Nucleus and Pontine Nuclei From the Cingulate Motor Cortex (M3 or Area 24a) in the Macaque Monkey. R.J. Morecraft1,2 and G.W. Van Hoesen3.1 Department of Anatomy and Structural Biology, 2Univ. of South Dakota, Vermillion, SD 57069 and Dept. of Anatomy2 and Neurology3, Univ. of Iowa, Iowa City, IA 52242.

The (ipsilateral corticostriate, corticorpulbro and corticopontine projections were studied in thirteen monkeys that received injections of tritiated amino acids into the cingulate (M3 or area 24a), supplementary motor (M1) and primary (M1) motor cortices. In M3 cases, light terminal labeling occurred over the head and anterior third of the caudate nucleus. Heavy patches were observed over the anterior pole and ventral portion of the putamen, then posteriorly labeling shifted in the dorsal portion of the red nucleus. In the red nucleus, labeling was found primarily over the ventromedial part of the parvicellular subdivision. At superior and mid-levels of the pons, labeling occurred over the ventromedial pontine gray matter. At inferior levels, patches of label formed an incomplete circle around the corticospinal tracts traversing the pons.

These data were compared to the distribution of projections from M1 and M2. Some topographic differences were found, but overall, the distribution overlap occurred as well. For example, the projection from M1 favored the dorsal motor part of the putamen, M3 the ventral motor part, and M2 an intermediate position. In the red nucleus, the densest distribution of labeled fibers terminated laterally from M3, medially from M3 and in between from M2. All three motor cortices projected heavily to the peripheral border of the medial and ventral portion of the basis pontis.

These observations show that M3 targets subcortical motor centers located at multiple levels of the neural axis. Judging from detailed comparisons, the highly interconnected cortical motor representations seem to be characterized as well, by overlap in their corticofugal projection zones. The terminal distribution of these pathways may provide opportunities of unity as well as diversity in terms of their influence on subcortical motor centers. (Support: NS 14944, USD Fac. Dev. Award)

It has been shown that electrical stimulation of the motor cortex in rats induces the expression of immunodetectable Fos-like protein (Fos-LI) in neostriatal neurons (Fu and R.M. Beckstead, Neuroscience 40(4):329-334). We have examined the effect of electrical stimulation of the motor cortex in awake and Nembutal-anesthetized rats (250 micro curies at 4 Hz of high frequency current pulses through parallel bipolar electrodes for 1 hr) on the induction of Jun B-like immunoreactivity (Jun B-LI).

We show that stimulation expressed only Jun-B-LI in the rat striatum in the same topographical location as Fos-LI-positive nuclei. However, with double-labeling techniques, we found that Jun B-LI and Jun B-LI were only partially colocalized, i.e., Jun B-LI was expressed only Fos-LI, some of Jun B-LI and both. This result suggests that striatal neurons respond to cortical stimulation with heterogeneous responses. Jun B-LI was the most visible signal detected in projection neurons expressing enkephalin or DARPP-32. We found Jun B-LI-positive nuclei in a low to moderate percentage of periventricular - ChAT and NADPH-diaphorase-positive interneurons. The presence of Jun B-LI in parvalbumin-positive, presumably GABAergic, immunoreactive neurons with the result obtained by chemical stimulation of the motor cortex (Berretta et al SNA 94). After which Jun B-LI was never seen in parvalbumin-positive neurons. Such specific differences in the patterns of neostriatal gene induction by electrical and chemical activation of the cortex may be the result of indirect antinomic effects of electrical stimulation. They might also reflect the differences in cortical activity produced by direct synchronous activation of cortical neurons by electrical stimulation, in contrast to release of endogenous cortical activity by removal of inhibition. Supported by NIH Javits Award NS25529.

We thank Dan R. Bravo, H. Hemmings, and P. Greengard for assistance.


Local pharmacological blockade of GABAergic transmission in the motor cortex awake rats was induced via chronic head-mounted wells (Berretta et al 94). This treatment evoked discrete body movements and, at 2 and 4 hours, induced the expression of IEGs in localized brain regions. We immunocytochemically characterized striatal neurons expressing Jun B, Jun B, and NGFI-A-like immunoreactivity (+LI) in the ipsilateral striatum. There was also a weak contralateral IEG induction. Large numbers of Fos- and Jun B-positive nuclei were in striatal neurons expressing enkephalin or DARPP-32, protein characteristic of striatal projection neurons. These results parallel similar findings in the same lesion model. Jun B-positive neurons were in a low to moderate percentage of periventricular - ChAT and NADPH-diaphorase-positive interneurons. The presence of Jun B-LI in parvalbumin-positive, presumably GABAergic, immunoreactive neurons with the result obtained by chemical stimulation of the motor cortex (Berretta et al SNA 94). After which Jun B-LI was never seen in parvalbumin-positive neurons. Such specific differences in the patterns of neostriatal gene induction by electrical and chemical activation of the cortex may be the result of indirect antinomic effects of electrical stimulation. They might also reflect the differences in cortical activity produced by direct synchronous activation of cortical neurons by electrical stimulation, in contrast to release of endogenous cortical activity by removal of inhibition. Supported by NIH Javits Award NS25529.

We thank Dan R. Bravo, H. Hemmings, and P. Greengard for assistance.

406.3 CORTICAL FOS EXPRESSION FOLLOWING DOPOAMINERGIC STIMULATION: DIFFERENTIAL DYE BIBLING AND ITS BREAKDOWN. G.J. Haacke†, D.N. Ruskie†, and J.P. Marshall†, Departments of Physical Medicine & Rehabilitation and Psychology, University of California, Irvine, CA 92697-4550.

While many recent studies have examined immediate-early gene expression in the basal ganglia following dopaminergic stimulation, few have focused on the cortical cortex, the prime target (via the thalamus) of basal ganglia output. We have examined the areal and laminar distribution of Fos immunoreactivity in the cerebral cortex of rats following systemic administration of dopaminergic agonists with particular attention to regions involved in decision-making regions and motor cortex. Combined, but not separate, D1 (SKF 38393 20 mg/kg) and D2 (quinpirole 3 mg/kg) agonist-stimulation induced pronounced Fos expression in frontal and parietal, but not occipital cortex in normal rats. Similarly, following amphetamine, cortical Fos could be blocked by either a D1 (SCH 23390, 0.5 mg/kg) or a D2 (clozapine, 0.5 mg/kg) antagonist. By contrast, following a regimen of reserpine (1 mg/kg/day for 5 days) that is known to cause a breakdown in D1/D2 synergism by behavioral and basal ganglia Fox measures, independent stimulation of D1 or D2 receptors resulted in cortical Fox expression. Irrespective of treatment condition, Fox expression occurred in a distinct laminar pattern that appeared to vary somewhat with cortical field. Dopaminergic modulation of cerebrocortical Fox may occur at the level of the striatum as part of a cortico-striato-pallido/igro-thalamo-cortical circuit; future studies will test this hypothesis. These findings show that the principles of D1/D2 synergism and its breakdowns apply to the stimulation of cerebrocortical Fox by dopaminergic agents. Furthermore, this Fox expression is correlated with motor behavior.

406.4 TRUNCATED FOsB IS RESPONSIBLE FOR THE LONG LASTING INCREASE IN STRIATAL FOS-LIKE IMMUNOREACTIVITY PRODUCED BY DOPOAMINERGIC DENERVATION. G.R. Redmond†, J.-P. Poulie*, B.T. Hop*, E.J. Nigel†, Y. Nakabolu, M.J. Iadarola*, N. Widel, and M. St.-Jean†, Dept. of Pharmacology, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5. Dept. of Psychiatry†, Yale University, 34 Park St., New Haven, CT, USA. Dept. of Biochemistry§, Medical Institute of Bioregulation, Kyushu University 69, Fukuoka 812, Japan. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD, USA.

Using an antibody raised against the DNA binding region of Fox, we have reported that destruction of the nigrostriatal pathway by injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle produces a long-lasting (>3 months) increase in striatal Fos-like immunoreactivity. In order to determine the nature of Fos immunoreactive protein(s) responsible for this increase, Western blots were performed on striatal extracts. Approximately six weeks after the 6-OHDA lesion, expression of a 38 kD Fos immunoreactive protein was dramatically enhanced. Since the molecular weight of the truncated form of FoxB (FoxB) is 35 kD, we examined FoxB and FoxBl expression using two antibody that recognize both FoxB and FoxBl while the other recognizes just FoxB. Western blotting and immune-histochemistry which demonstrated that the increase occurred exclusively in medium-sized neurons of the denervated striatum. These results suggest that FoxB may participate in those intracellular events which maintain altered neuronal gene expression in the striatum after dopaminergic denervation. (Supported by grant MT-11539 from the MRC of Canada).


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406.6 EFFECTS OF METHYLXANXV-METHANOL ACETATE ON THE FORMATION OF STRIATAL COMPARTMENTS. W. Wudhaw*, M. Cinimino*, F. Caterella, B.A.Flumerfelt, and C.C. Neug. Dept. of Anatomy, University of Western Ontario, London, Canada, N6A 5C1 and Institute of Pharmacological Sciences, School of Pharmacy, University of Milan, Via Balzaretti 9, Milaan, Italy, 20123.

The striatum can be segregated into two compartments, the patch and matrix, based on afferent and efferent projections and certain neurochemicals (eg. calbindin, somatostatin). Immunocytochemical studies indicate the neurons which comprise these two populations arise at two different time points from separate populations of developing neurons. Neurons destined to be localized within the patch are born between E10 and 14 while matrix neurons are born between E16 and E18. In order to attempt to knockout the patch or matrix compartments, methylxanxv-methanol acetate (MAMA) was injected intraperitoneally (25mg/kg) into E13 or E17 pregnant rats. MAMA is a potent toxin which kills dividing neuroblasts. It is effective for 24hrs. post-injection with effects peaking after 8hrs. Pups obtained from the pregnant mothers were allowed to grow to adult age. Western blotting was performed on a number of proteins in both the whole brain and the striatum. The results indicate that calbindin and somatostatin are decreased while the density of the glutamate receptor is increased. The results also suggest that MAMA injections at the appropriate developmental times can alter the phenotype. The ratio of compartment ratio. Research supported by NATO grant 900303, MRC and Huntington's Society of Canada.

We have used in situ nick transfer to identify increases in DNA strand breaks in individual neurons of the striatum after local injections of a radioactive agonist (NMDA, 2 mg/kg, IP) or a antagonist (kainic acid, 2 mg/kg, IP) into the striatum, 24 hrs later. We report that in situ nick transfer detects DNA strand breaks in neurons, and that the number of breaks is proportional to the intensity of neuronal injury, with the greatest damage occurring at the lesion center, where neuronal loss is greatest. Our results suggest that in situ nick transfer can be used to assess DNA damage in individual neurons, and that it may provide a sensitive and specific measure of neuronal injury.


The globus pallidus (GP), one of the main output nuclei of the striatum, receives an inhibitory GABAergic projection from the striatum and an excitatory glutamatergic input from the subthalamic nucleus. Lesions of the GP led to decreases in the expression of GAD 67 mRNA in the GP, but not in the GPi or SNr, suggesting that the decrease in GAD 67 mRNA levels in the GP is not due to an increase in excitatory input from the subthalamic nucleus.


We have shown that loss of immunoreactivity for the immunoassay, highly polialysinated form of the Neural Cell Adhesion Molecule (PSA-NCAM-IR) occurs at the end of the postnatal period in rat striatum (Szele et al., Neurosci., 1994). Ultrastructural studies show that, at postnatal day 7 (P7), PSA-NCAM-IR is associated with both axonal growth cones, and with the cytoplasmic membrane of immature striatal neurons. At postnatal day 18, PSA-NCAM-IR was still associated with intracellular striatal neurons, but labelling of the cytoplasmic membrane was much more diffuse than at P7. In addition, PSA-NCAM-IR immunoactivity was associated with both pre and post-synaptic elements of asymmetrical synapses on dendritic spines. The results confirm that during late postnatal development, PSA-NCAM-IR is still associated with striatal neurons, as well as with synaptic inputs, likely originating from the cerebral cortex and/or the thalamus. The second experiment examined the role of PSA-NCAM-IR in the striatum in the development of the nigrostriatal dopaminergic pathway by bilateral injections of 6-hydroxydopamine in the striatum at P2 resulted in a profound loss of tyrosine hydroxylase immunoreactivity in the striatum, but did not affect the time course of loss of PSA-NCAM-IR. In contrast, in weaver mice, which exhibit a profound loss of dopaminergic inputs to the dorsolateral striatum postnatally, the loss of PSA-NCAM-IR in the striatum was delayed by approximately 1 week compared to wild type mice. These results suggest that dopaminergic inputs do not play a critical role in the shift from immature to mature forms of NCAM in the striatum, and that other factors than the loss of nigrostriatal neurons play a role in the delayed maturation of PSA-NCAM in weaver mice. Supp. by NS29230.


The mRNA levels encoding for the two isoforms of glutamate decarboxylase (GAD65 and GAD67) were measured in different sectors of the caudate and putamen in the internal (GPe) and external (GPi) segments of the pallidum in control (n=5) and parkinsonian (n=7) monkeys (Saimiri sciureus) after administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Cresyl violet sections of the brains were processed for in situ hybridization in situ histochemistry with 35S labelled cRNA probes and the radioautographic labeling was quantified on X-ray films or emission radiography. The mRNA levels of dopaminergic fibres were measured after 3H-mazindol binding and was shown to be widespread throughout the ventral and the caudate. The expression of GAD65 mRNA levels is increased in the ventral putamen. In MPTP-treated monkeys, both GAD65 and GAD67 mRNA levels were increased in both the dorsal and ventral putamens and the caudate. The increase in GAD65 mRNA levels was significant only in the ventral putamen. In MPTP-treated monkeys, both GAD65 and GAD67 mRNA levels were increased. The present results demonstrate that gene expression of both GAD isoforms is significantly altered throughout the basal ganglia. In the striatum, increased GAD65 and GAD67 expression appears prominent in somatodendritic territories such as defined by cortical afferents. The results also support the hypothesis of an increased activity of striatal and pallidal output GABAergic neurons in parkinsonism (Supported by FRQs, the Parkinson Foundation of Canada and NSERC-0155607).


The role of D1 and D2 dopamine receptor subtypes on the regulation of neuronal GAD isoforms was studied using the two isoforms of glutamate decarboxylase (GAD65 and GAD67) and pterephenyl 1H-pyrene (PPE) ion in striatum was investigated. The mRNA levels encoding for GAD65, GAD67 and PPE were determined by in situ hybridization histochemistry and quantified by computerized densitometry. Chronic treatment (10 days) with the D1/D2 dopamine receptor agonist apomorphine or the D1 agonist SKF 38393 significantly increased the mRNA levels of both GAD65 and GAD67 in the striatum. Chronic treatment (10 days) with the D2 dopamine receptor agonist quinpirole decreased both GAD65 and GAD67 mRNA levels in the striatum. Chronic blockade (4 days) of D1 and D2 dopamine receptors with haloperidol or D2 dopamine receptor antagonist SB 27706 and PPE did not change GAD65 and GAD67 mRNA levels. Chronic blockade (14 days) of D1 receptors with SCH 23390 induced a slight decrease in PPE mRNA levels. These results show that gene expression of each isoform of GAD is differentially regulated in the rat striatum. Gene expression of DOP-AP seems to be under the excitatory control of D1 dopamine receptors. These results also suggest that each GAD plays a distinct role in the regulation of GABA synthesis in striatum (Supported by FRQs, Parkinson Foundation of Canada and NSERC-0155607).

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406.13 INTRASTRIAL INJECTIONS OF QUINOLINIC ACID SHOW MARKED CHOLINE ACETYLTANSFERASE AND NADPH-DIAPHORASE CELL LOSS. S.M. Walker, W.C. Perryman, and G.K. Riecker*. Department of Anatomy and Cell Biology, School of Medicine, University of North Dakota, Grand Forks, North Dakota.

The debate as to whether the striatal pathology observed in the Quinolinic Acid (QA) model of Huntington's Disease (HD), as it demonstrates a selective sparing of aspiny interneurons, is unresolved. QA is an endogenous, excitatory amino acid that is known to spare fibers of passage. It is the intent of this study to define cell types and the associated loss within the HD model. Alzet mini-osmotic pumps were utilized for a 14 day, 340 Mg/mL, Ph 7.8, QA 0.5 % per hour injection period. Choline Acetyltransferase (Chat) immunohistochemistry and NADPH-diaphorase histochemistry were used to determine cell spacing and cell loss of Chat and NADPH cell counts were determined in adjacent sections. The results indicate marked cell loss. There is an overall 27% decrease in diapomorphic-positive cells and a 36% decrease in Chat-positive cells. Thus, preliminary results suggest a significant loss of intrastriatal Chat and NADPH aspiny interneurons. Supported by BRSG.

406.14 LOCALIZATION OF "FLIP" AND "FLOP" SPICE VARIANTS OF AMPA-SELECTIVE GLUR2 AND GLUR3 EXCITATORY AMINO ACID RECEPTOR SUBUNITS ON STRIATAL PROJECTION NEURONS. S.L. Tallaksen-Greene* and R.L. Albin*. Department of Neurology, University of Michigan, Ann Arbor, MI 48109.

We have previously shown that the majority of striatal projection neurons are immunostained using an antibody recognizing an epitope conserved on Glur2/3, 1 and 4c subunits and common to both "Flip" and "Flip" splice variants. In order to determine the relative expression of Glur2 and 3 splice variants on striatogiral and striatonigral neurons we used in situ hybridization in combination with the retrograde transport of the fluorescent tracers Fluoro-Gold (FG). Unilateral injections of FG were carried out into the substantia nigra or globus pallidus of adult male rats. After 12 days, the animals were killed by decapitation. Twelve pm sections were processed for in situ hybridization using 35S-labeled oligonucleotide probes specific for Glur2 and Glur3 mRNA. These findings indicate that splice variants of AMPA receptor subtypes are differentially expressed by striatal projection neurons. Supported by NS19613 and the Huntington's Disease Society of America.


In Parkinson's disease (PD), the striatal dopaminergic denervation induces complex changes in the functional circuitry of the basal ganglia which result, ultimately, in an oversupply of the output nuclei, substantia nigra pars reticulata (SNr) andsubstantia nigra pars compacta (SNc). The high levels of NADPH-diaphorase activity, supporting the presence of NOS containing fibers in the basal ganglia. The aim of this study was to investigate mitochondrial enzyme activity, a reliable marker for synaptic activity, in different nuclei of the basal ganglia of rats following a selective stereotaxic lesion of the nigrostriatal pathway or the STN. For this purpose, a group of rats received a unilateral k-hydroxydopamine lesion of the substantia nigra pars compacta and medial forebrain bundle, whereas another group had the STN selectively ablated by means of an injection of 25 mmoles of N-methyl-D-aspartate. Sulfate dehydrogenase and cytochrome oxidase activities were assayed histochemically on brain sections previously mounted on polylysine-coated slides. A densitometric comparison between the enzymatic staining in the lesioned and the unlesioned side was then carried out.

The rats with the nigrostriatal deploration showed, ipsilaterally to the lesion, an increased enzymatic activity in the projection nuclei of the STN, SNr, entopeduncular nucleus, and globus pallidus (the rodent homologues of MOP and lateral globus pallidus, respectively). Conversely, the enzymatic activity in these areas was reduced, ipsilaterally, by the complete ablation of the STN. These data lend further support to the more recent view of the basal ganglia circuitry organization focusing, in particular, on the modulatory activity that the SNr neurons, excitatory (glutamatergic) in nature, play in the regulation of the basal ganglia output.

406.16 DECREASED NUMBER OF SOMATOSTATIN mRNA CONTAINING NEURONS IN THE SUBSTANTIA GANGLIAOF STEREOELECTRODE TREATED RATS. D. Høyer-K* F. Andersen, O A. Christensen, B. Westm, and B. Jensen. Ha. 1 Pharmaceutical Department, University of Odense. 2Department of Neurological Research Laboratory, University of Aarhus, Denmark. 3Department of Physiology, University of Bergen, Norway.

For the first time and by stereological cell counting, it has been possible to demonstrate a reduction in the number of somatostatin (SS) mRNA containing neurons in the dorsal striatum of rats with dyskinetic symptoms caused by long-term treatment with a dopamine receptor blocking drug. Adult rats were treated with haloperidol for 6 months and the behaviour video-taped. Five rats which developed severe dyskinesia symptoms and five rats, which did not, together with five control rats were anesthetized and perfused. One series of vibratome sections of striatum were used for the in situ hybridization with a SS-mRNA probe. A second series of sections were labeled oligonucleotide probe. The total number of neurons expressing SS mRNA in the dorsal striatum was estimated with the optical fractionator technique. The mean number of SS mRNA containing cells in the treated rats with dyskinetic symptoms was significantly less than for the rats with no symptoms and the control rats.

406.17 EXPRESSION OF METABOTROPIC GLUTAMATE RECEPTOR MIGLURS BY SPECIFIC RAT STRIATAL CELL POPULATIONS. C.M. Tsai*, D.G. Standaert, G.B. Landwehrmeyer, J.B. Penney, and A.B. Young. Department of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Glutamate has a central role in basal ganglia regulation of motor behavior, and is the main afferent neurotransmitter to the striatum. Glutamate acts via many different striatal receptors and thereby increase thalamic feedback to the striatum. Although amphetamine alone was without effect, the combination of amphetamine plus MK-801 produced a large increase in striatal ACh concentrations. These results suggest that the effects of dopaminergic agents on the striatal cholinergic interneurons are a composite of the direct striatal effect of such drugs and of their ultimate effect on the glutamatergic thalamosomal feedback loop.

406.18 INTERACTION OF DOPAMINERGIC AND GLUTAMATERGIC SYSTEMS IN THE REGULATION OF STRIATAL CHOLINERGIC ACTIVITY. Christopher J. Schmidt* and Volli L. Taylor, Marion Merrifell Doh Research Institute 2110 E. Galbraith Road, Cincinnati, OH 45215.

The balance between dopaminergic and glutamatergic activity within the cortico-striatohamal pathway (CST) is an important determinant of sensory input through the thalamus. Disruptions of this balance in favor of glutamate or dopamine are believed to produce the symptoms of Parkinson's disease or schizophrenia, respectively. The cholinergic interneurons of the striatum are regulated by long loop feedback loops involving dopaminergic and glutamatergic afferents from the midbrain and the thalamus, respectively. The strategic position of these neurons allows changes in tissue concentrations of acetylcholine (ACh) to be used to assess effects of dopaminergic and glutamatergic agents on striatal function and potentially the CST pathways. Consistent with a reduction in cholinergic striatal activity, high doses of the uncompetitive NMDA antagonist MK-801 produced a modest accumulation of striatal ACh while the D2 agonist quipride produced a robust increase in tissue concentrations of the transmitter. Pretreatment with quipride augmented the elevation of ACh produced by MK-801 in a synergistic fashion. The D2 antagonist, SCH 23390 alone produced only a small increase in ACh yet completely prevented any further elevation of ACh by MK-801. The D2 antagonist haloperidol produced the expected decrease in striatal ACh and also completely blocked the effect of MK-801. Thus inhibition of cholinergic striatal activity with the D2 agonist potentiated the effect of MK-801 whereas treatments which increase striatal/cholinergic activity prevent any further effect of MK-801. These observations suggest that glutameric feedback from the thalamus may be important in determining the magnitude of the MK-801 effect. To test this hypothesis, amphetamine was used to enhance thalamic activity and thereby increase thalamic feedback to the striatum. Although amphetamine alone was without effect, the combination of amphetamine plus MK-801 produced a large increase in striatal ACh concentrations. These results suggest that the effects of dopaminergic agents on the striatal cholinergic interneurons are a composite of the direct striatal effect of such drugs and of their ultimate effect on the glutamatergic thalamosomal feedback loop.

406.19 EVIDENCE FOR NOSPECIFIC STIMULATION OF THE STRIATUM BY STIMULUS-LOCKED MICROSTIMULATION. R. Kergues. Department of Neurology, Massachusetts General Hospital, Boston, MA 02114.

We used a double label in situ hybridization technique to determine if substrates of proneural differentiation could be used to identify neurons with this technique. In situ hybridization results suggest that the use of a double label in situ hybridization technique to determine if substrates of proneural differentiation could be used to identify neurons with this technique.

The expression of m1 and m4 muscarinic receptor mRNAs in the rat dorsal striatum was examined using in situ hybridization histochemistry. The results indicate that m1 and m4 muscarinic receptor mRNAs are expressed in the striatum of the adult rat.

Low vs. High Dose and Acute vs. Subchronic Haloperidol Treatment Induces Different Patterns of Immediate-Early Gene Expression in the Striatum, B. Eibl, E. Pellecchia, N. Hu, and A.M. Gazdzik. Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA 02139.

The extrapallidal side effects of haloperidol therapy are thought to be related, in part, to its modulation of striatal activity by antagonism of D2-like dopaminergic receptors. Consistent with such striatal effects, neural activity mapping with immediate-early gene expression immunohistochemistry has shown that typical neuroleptics, more than eticlopride, induce IEGs in the mesencephalon. We have examined the effects of repeated haloperidol exposure on regulation of members of the Fos family in the adult rat (Pax, Fos, Fra, Jun, Elk, and Dusp).

Blockade of NMDA and Muscarinic Receptors in the Nucleus Accumbens Causes Mice to Rotate in Opposite Directions, A. Svensson*, M.L. Carlsson, and A. Carlsson. Department of Pharmacology, University of Göteborg, 5-413 90 Göteborg, Sweden.

The effects on rotational behaviour of glutamate blockade and acetylcholine blockade in the nucleus accumbens of male NMRI mice with different doses in the dopaminergic system.

We have previously shown that a unilateral injection into the nucleus accumbens of an NMDA receptor antagonist (AP-5) causes the animals to rotate. The rotation is predominantly ipsilateral in animals with intact dopaminergic systems. In the present study, dopamine-pretreated mice were treated with AP-5, or with control saline, and the direction of rotation assessed. AP-5 produced a marked decrease in rotational behaviour in the lesioned side, indicating a decrease in dopamine release.
REGULATION OF Na⁺/K⁺-ATPase ACTIVITY BY PROTEIN KINASE C PHOSPHORYLATION IN RAT NEOSTRIATUM. G.L. Spector,1 E. Fiance,1 A. Nishi,1,2 M. J. Caplan,2 A. Apetera3 and P. Greenough1 1Lab. of Mol.
and Cell. Neurobiology, Rockefeller Univ., New York, NY 10021; 2Dept. of Pediatrics, Kurume Univ. School of Medicine, Kurume, Japan; 3Dept. of
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The ion pump Na⁺/K⁺-ATPase is a ubiquitous integral membrane protein formed by a catalytic subunit and a β subunit, which transports Na⁺
from the inside to the outside of the cell, in exchange for K⁺. This enzyme is of fundamental importance in ionic balance and maintaining the electro-
chemical gradient across the plasma membrane underlying resting and action potentials in neurons. Previously, we showed that, in vitro, phosphorylation of the catalytic subunit (α subunit) of Na⁺/K⁺-ATPase is increased in two CAMP-dependent protein kinase or protein kinase C (PKC) significantly enhances its activity. Three different isoforms of Na⁺/K⁺-ATPase have been identified in neurons, their distribution differing in the various brain regions. We detected both α1 and α3 isoforms in primary cell cultures from rat striatum. We found that addition of the activator of protein kinase C, phorbol 12,13-dibutyrate (PDBu) (5 μM), to α3 pre-labelled slices from rat striatum stimulates by several-fold the phosphorylation of the α1 subunit and also increases the phosphoryl-
ation of the α3 subunit. In agreement with the data obtained in vitro, treatment of striatal neurons with PDBu inhibited Na⁺/K⁺-ATPase activity by 25%. These data provide evidence for regulation of Na⁺/K⁺-
ATPase activity, and hence neuronal excitability, by PDBu phosphory-
ation in the striatum. [Supported by USPHS grant MH 48099].

407.11 IMMUNOCYTOCHEMICAL PROPERTIES OF RAT CAUDATE-PUTAMEN NEURONS EXPRESSING DOPAMINE-
MODULATED K⁺ CHANNELS, Gabriela J. Green,3 Yong-Jian Lin, June-Chih Liu, Barbara I. Wassef, and Jonathan E.
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Boston, MA 02115.

We have previously used patch-clamp recording to describe an 85 pS K⁺ channel which is modulated by D₂-like dopamine recep-
tors on freshly dissociated rat corpus striatum neurons. We are
now using some cytochemical methods to identify the cells expressing this channel. Using phase-contrast and immunocyto-
chemical techniques we were able to detect an immunoreactive cell in the medial, intermediate and lateral regions of the caudate-putamen. This cell type appeared to be pyramidal-like in shape and was stained more darkly in the lateral than the medial region. This cell is characterized by being immunopositive for choline acetyltransferase and GABA, and negative for glutamic acid decarboxylase. It was located in the striatum and is consistent with the D₂-like dopamine receptor on this cell being stimulated by the administration of dopamine. The findings presented here are consistent with the idea that this cell type is a putative projection neuron which is part of the basal ganglia. [Supported by M01-RR02414, the National Institute of Neurological Disorders and Stroke, and The Uehara Memorial Foundation].

407.12 GAT-1 GABA TRANSPORTER mRNA IN RAT BRAIN: CELLULAR CO-
EXPRESSION WITH GAD67 mRNA AND PARVALBUMIN mRNA. S.J. AUGOOD,3 K. WESTMORE AND P.C. EMSON, MRCP Molecular
Neuroscience Group, Dept. of Neurobiology, BBSRC Babraham Institute, Cambridge CB2 4AT, U.K.

The re-uptake of GABA into pre-synaptic nerve terminals terminates GABA signalling. Several GABA transporters have been cloned including the GABA transporter GAT-1, which displays micromolar affinity for GABA when transfected in vitro (1.3). It has been suggested that cells which tonically release GABA may utilise more GAD67 than GAD65 enzyme. The expression of GAT-1 mRNA is upregulated in vivo by several factors (2). The expression of the calcium-binding protein parvalbumin (PV) is associated with fast-firing cells in the rat brain. This study aimed to determine if GAT-1 mRNA was expressed by GABA cells, and if PV cells represented a sub-
group of GAD67-GAT-1 cells. Cellular sites of (i) GAD67 and GAT-1 mRNA, and (ii) GAD67 and PV mRNA were visualised using a combination of alkaline phosphatase (AP), and 35S-labelled oligonucleotides. Strong hybridization signals for these three probes were detected in cells within most brain regions including the cerebral cortex, basal ganglia, hippocampus and cerebellum. Examination of silver grain deposits overlying AP-positive cells showed that most high-GAD67 cells were co-labelled for the GAT-1 mRNA, although the expression of GAT-1 mRNA was widespread: a divergence of the two signals was seen in the reticular thalamus and inferior colliculus. PV mRNA was detected in some high-GAD67 cells. Detailed analysis of the striatum will be presented. These data suggest strongly that GAT-1 is a pre-synaptic marker of most high-GAD67 GABA cells in vivo and that tonically active stratal GABA/PV cells may utilise GAT-1 for re-uptake. (1) Borden et al., 1992 J Biol Chem 267:21086-21104; (2) Elands et al., 1991 Neuron 7:91-100; (3) Guastella et al., 1990 Science 250:1300-6. SJA is a Wellcome Trust Mental Health Research Fellow.
407.1
PARTIAL LESIONS OF THE NIGROSTRIAL DOPAMINE PATHWAY ALTER SUBSTANCE P BUT NOT ENKEPHALIN mRNA IN THE RAT STRIATUM. L.K. Nicklas, M. A. Kulkoski, K.J. Liff, and N.G. Gerfen, Department of Anatomy/Neurobiology and Pharmacology, Univ. of Tennessee, College of Medicine, Memphis, TN 38133, and the S. Jackson, Dept. of Neurology, C. R. Keefe, Section of Neuroanatomy, LCB, NIMH, Bethesda, MD 20892.

The expression of enkephalin and substance P mRNA is regulated by the nigrostriatal dopamine (DA) pathway. For example, near total depletion of striatal DA levels results in an increase in enkephalin and a decrease in substance P mRNA. However, it is unknown whether partial lesions of striatal DA content produce similar changes in the respective mRNAs. To test whether compensations in DA synthesis and release follow partial DA depletion prevent the lesion-induced alterations in enkephalin and substance P mRNA, varying concentrations of 6-OHDA were injected unilaterally into the substantia nigra. Seven days after injection of 6-OHDA (1-16 μg) in vehicle, in situ hybridization was employed to examine tyrosine hydroxylase mRNA in the substantia nigra and enkephalin and substance P mRNA in the striatum. The extent of the DA depletion was determined by measuring striatal DA tissue content. Subjects were divided into three groups based on the extent of striatal DA depletion: <50%; 50-90%; and >90%. The decrease in tyrosine hydroxylase mRNA closely paralleled the changes in striatal tissue DA content in all groups. Although no significant change in substance P mRNA was detected in rats with <50% DA depletion, a 24% and 68% decrease was observed in the 50-90% and >90% depleted groups, respectively. In contrast, a significant increase in enkephalin mRNA was not detected until a >90% depletion of striatal DA was produced. In summary, alterations in tyrosine hydroxylase mRNA in the substantia nigra are well correlated with decreases in striatal tissue DA levels. In addition, whereas partial lesions of the nigrostriatal DA pathway produce a decrease in substance P mRNA, a near total depletion of striatal DA levels is necessary to increase enkephalin mRNA. Supported by this grant NS26471, the United Parkinson Foundation and the Human Frontiers Program.

407.15
DIFFERENTIAL INVOLVEMENT OF NMDA RECEPTORS IN STRIATUM IN D1-DOPAMINE RECEPTOR-MEDIATED BEHAVIOR AND IMMEDIATE EARLY GENES (IEGS). K.A. Kenealey and C. R. Gerfen, Section of Neuroanatomy, LCB, NIMH, Bethesda, MD 20892.

Dopamine's effects in striatum often are thought to result from its ability to modulate the response of striatal neurons to other afferents. For example, activation of D1 dopamine receptors increases conductance through the functional subunit of glutamate receptors. D1-receptor activation also increases expression of the immediate early genes zif268 and c-fos in striatum. To further understand interactions between dopamine and glutamate through the regulation of striatal function, we examined the contribution of NMDA receptors to immediate early gene expression in the dopamine-depleted striatum by infusing NMDA receptor agents into the striatum of freely moving rats. Experiments determined if changes were blocked by in situ hybridization histochemistry. Infusion of NMDA (1 mmol) for 20 min blocked the contralateral rotation and decreased the expression of c-fos induced by the D1 agonist SKF 38393 (2 mg/kg, ip). CPP did not, however, affect SKF 38393-mediated induction of zif268 under the same conditions. The data indicate that D1-mediated changes in striatal output are dependent, at least in part, on ongoing excitatory input to the striatum, as evidenced by CPP blockade of D1-induced contralateral rotation and its partial reduction of D1-mediated c-fos induction. However, D1-mediated gene regulation in these neurons is not completely dependent on NMDA receptor activity, as evidenced by the incomplete blockade of c-fos induction and lack of effect on zif268 induction.

407.16
DYNOPHIN OPIOID INHIBITION OF D1 Dopamine Receptor-MEDIATED INDUCTION OF IMMEDIATE-EARLY GENES IN THE STRIATUM. H. Stein and C. R. Gerfen, Section of Neuroanatomy, NIMH, Bethesda, MD 20892.

Dynorphin is an opioid peptide contained in striatognal projection neurons. In such neurons dopamine agonists produce rapid induction of immediate-early genes (IEGs), such as c-fos and zif 268. Recent studies showed that IEG induction by the indirect dopamine receptor agonist cocaine in striatum is inversely related to striatal dynorphin expression, and (2) can be suppressed with systemic and infrastriatal administration of the dynorphin (kappa opioid receptor) agonist spiradoline. These results suggest that dynorphin is involved in the regulation of dopamine input to striatognal neurons, directly and/or indirectly, through kappa opioid receptors located in the striatum. In the present study, we examined whether dynorphin exerts direct influence on striatognal neurons by analyzing the effects of the dynorphin agonist spiradoline (1-10 mg/kg) on IEG induction by stimulation of D1 dopamine receptors which are expression in these neurons. Gene expression was assessed with in situ hybridization histochemistry. Spiradoline partially suppressed IEG induction by the selective D1 receptor agonist SKF 38393 in the dopamine-depleted striatum, while the lesser IEG response in the intact striatum was completely blocked. Striatal regions with higher levels of dynorphin/kappa receptor expression (ventral regions) showed greater inhibition of IEG induction than striatal regions with lower levels of these mRNAs (dorsal striatum). These results suggest that kappa opioid receptors on striatognal neurons participate in dynorphin-mediated inhibition of dopamine input to these neurons.

CONTROL OF POSTURE AND MOVEMENT VI

408.1
CHARACTERISTICS OF THE REPRESENTATION OF HAND IN SPACE FOR 3-D TACTILE LOCALIZATION. P. Dassonville* and A. P. Georgopoulos, Minneapolis VMMC Brain Science Center and Physiology Dept., University of Minnesota, Minneapolis, MN 55455.

To encode the location of a tactile stimulus in 3-D space, information of the stimulus' somatotopic location must be combined with a representation of limb positions. Previous studies investigated the time course of the implementations of hand space by instructing subjects to point, in complete darkness, to the 3-D location of a tactile probe presented to the fingertip during a previous arm movement (Dassonville et al, 1993). Our study evaluated the probe to be at the locations occupied by the hand approximately 100 ms after probe onset, indicating that the dynamic representation of hand in space does not compensate for counterclockwise, kinesthetic, and/or motor factors. However, this representation is seen in some subjects with a velocity different from that of the hand itself.

In the present study, two additional problems were investigated as follows: (1) Subjects performed a 3-D localization task under normal lighting. The pattern of errors observed in each subject was similar to that observed when tested in complete darkness. Thus, the internal representation of hand in space does not have an appreciable visual component. (2) Four subjects were instructed to simulate whether the probe was presented before, during, or after the movement. Probes presented <38 ms before movement onset were perceived as occurring during the movement, whereas those presented >145 ms before movement termination were perceived as occurring after the movement. Across subjects, these temporal shifts were highly correlated with the temporal shifts measured in the original localization task. Thus, the subjects' perception of movement onset and termination appears to rely on the same internal representation of hand that is used for 3-D tactile localization.

408.2
REACHING ERRORS RESULTING FROM THE DEGRADATION OF AN INTERNAL MODEL. C. A. Bunse*, J. Bolte, J. F. Schoetig, and R. E. Poppele, Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55455.

We investigated, by means of simulations, possible mechanisms responsible for the systematic directional errors exhibited by deafferented patients during reaching movements (Ghez et al, 1990). Two aspects of altered feedback control were evaluated: the inability to sense initial conditions and the degradation of an internal model. The first simulation demonstrated that the pattern of directional errors exhibited by deafferented patients could not be explained by assuming inadequate information regarding initial arm configuration. In contrast, a second simulation, which introduced random errors in torque production, produced results that corresponded closely to the general pattern of errors exhibited by deafferented patients. We conclude that these directional errors do not result simply from a failure to compensate for inertial anisotropies, but are consistent with a degradation of the sensory feedback which leads to an increased variability in the mapping between joint torques and motion.
408.3 SHORT-LASTING CONTROL SIGNALS UNDERLIE THE FASTEST SINGLE-JOINT MOVEMENTS. A.G. Feldman, S.V. Adamovich, R. Forget*, M.F. Levin, Research Ctr., Rehabilitation Inst. of Montreal, Quebec, Canada H3S 2V4

We tested two versions of the hypothesis that movements are produced by two motor systems in the CNS: 1) that shifts end near the peak velocity of movement and 2) that shifts proceed until the end of movement. The first predicts that movement time may be significantly reduced by opposing loads without changes in the control pattern. Subjects flexed their elbows (and performed control trials) and produced two test trials; movements were opposed by loads generated by a torque motor. Subjects had no visual feedback and were instructed not to correct arm deflections when perturbations occurred. At the end of the movement, the load was removed leading to a secondary movement to the same final position as that in control trials (equifinality). The static arm positions before unloading were related to load torques. This finding and equifinality implies that subjects could reproduce the same control patterns regardless of perturbations. Test movements opposed by a high load ended near the peak velocity of control movements. Phasic and tonic EMG patterns were load-dependent. Results indicate that the control pattern is of short duration which suggests that rather than being pre-programmed, EMG signals represent long-lasting dynamic responses of the system to the short-duration control pattern, external forces and proprioceptive feedback.


Recent studies (Goodon et al 1993, Thach et al 1992) support Gordon Holmes' observations that cerebellar lesions affect simple movements at one joint and compound movements across several joints, the latter are affected disproportionately. From a mechanical standpoint, a multijointed movement consists of a summation of single-joint movements due to interaction torques (i.e., inertial, centripetal, and Coriolis) generated by one limb moving on the other. In this study we investigated if cerebellar subjects performing two-joint reaching movements under two conditions. For the "accurate" condition, seated subjects were asked to make a self-paced reach to touch a 1 cm target without any visual information. For the "fast" condition, seated subjects were instructed to move as fast as possible and touch any part of the 4 cm ball. Subjects were videotaped with markers at the index finger, shoulder, elbow, and wrist joints. Marker positions were digitized from the video recordings and used to calculate... Inverse dynamic equations (Soechting and Lacquaniti 1981) were used to estimate 1) net torques and 2) interaction torques at the elbow and shoulder joints. Preliminary data indicate that, under the "accurate" condition, cerebellar subjects moved in a manner that reduced the complexity of torques by decomposing the reach and/or slowing it down. Slowing the reach also permitted use of peripheral feedback to help shape the ongoing movement. Under the "fast" condition, cerebellar subjects produced a large number of interaction torques and often overshot the target. Fast reaching movements increased the magnitude of interaction torques (Soechting and Lacquaniti 1981) and normally require subjects to account for them in a predictive manner. We are currently addressing whether abnormalities in the "fast" movements made by cerebellar patients reflected the inability to account for only interaction torques or increasing magnitudes of all torques. We speculate that the cerebellum plays a role in generating appropriate commands to account for the complex nature of the torque components during fast, multijointed movements (NIH grant NS121777).

408.7 COORDINATION OF PLANAR TWO-JOINT ARM MOVEMENTS IN DIFFERENT DIRECTIONS. N. Vlot, D.B. J.D. Cooke, Faculty of Applied Health Science, University of Western Ontario, London, Canada N6G 1H1

In order for coordinated movement to occur, it is generally assumed that the CNS must somehow play an active role in counteracting the effects of interactional torques. Very few studies have directly examined the influence of such torques and the identified movement characteristics that are preserved during compensatory coordination during two-joint movements. We examined EMG-movement relations during planar elbow and wrist movements in which the two joints moved in different directions. i.e. elbow flexion and wrist extension at each joint. Elbow and wrist movements of different amplitude combinations were performed during a visual, stop-tracking task in which subjects were specifically required to attend to the horizontal and vertical angles at each joint. Elbow kinematics were generally unaffected by concurrent wrist movement. In contrast, wrist movement trajectories were variable and wrist movement coupling was observed. Qualitative changes were also observed in the pattern of muscle activation at the wrist joint. Specifically, wrist antagonist activity preceded wrist agonist activity in conditions when interactional torques could potentially produce a movement larger than required wrist movement. The variability observed in wrist trajectories suggests that the CNS may not play a direct role in the control of wrist movement. Rather, the observed kinematic and EMG changes at the wrist joint appear to be the direct result of a strategy used by the CNS to compensate for reaction torques resulting from elbow movements, ensuring the production of movements of designated amplitudes.

408.8 DELAYED VISUAL INFORMATION SLOWS DOWN THE TIME COURSE OF PRISM ADAPTATION IN HUMAN. S. Kingma, T. Kobay and T. Uka, Neuroscience Section, Electroclinical Laboratory, Tokushuk, 290, JAPAN

Accurate pointing is generally disturbed when the visual field is displaced by prisms, but gradually recovers. To test if the manner is disrupted by visual error signals that correlate in the error component with the motor output, the rate of prism adaptation was studied with verbal information. Nine subjects were trained to point rapidly at a target that appeared randomly in a square area (40x40 mm) on a target screen (400 mm away). Vision of the hand and the arm was always blocked during the movement by a liquid-crystal shutter, and an error signal was shown to the subject by the target light. The error signal was delayed by 200 ms in the visual displacement. Elbow and wrist movement trajectories were variable and wrist movement coupling was observed. Qualitative changes were also observed in the pattern of muscle activation at the wrist joint. Specifically, wrist antagonist activity preceded wrist agonist activity in conditions when interactional torques could potentially produce a movement larger than required wrist movement. The variability observed in wrist trajectories suggests that the CNS may not play a direct role in the control of wrist movement. Rather, the observed kinematic and EMG changes at the wrist joint appear to be the direct result of a strategy used by the CNS to compensate for reaction torques resulting from elbow movements, ensuring the production of movements of designated amplitudes.

408.9 ADAPTIVE CHANGES IN TORQUE CONTROL DURING REACHING OF YOUNG INFANTS. J. Konczak*, M. Brodt, J. A. Dichgans. Dept. of Neurology, Univ. of Tübingen, 72076, Tübingen, Germany. (Spon. EBSB)

When newborns attempt to produce goal-directed arm movements they are faced with the problem to actively control the forces for the fastest elbow flexions in humans: 1) that shifts end near the peak velocity of movement and 2) that shifts proceed until the end of movement. The first predicts that movement time may be significantly reduced by opposing loads without changes in the control pattern. Subjects flexed their elbows (and performed control trials) and performed two test trials; movements were opposed by loads generated by a torque motor. Subjects had no visual feedback and were instructed not to correct arm deflections when perturbations occurred. At the end of the movement, the load was removed leading to a secondary movement to the same final position as that in control trials (equifinality). The static arm positions before unloading were related to load torques. This finding and equifinality implies that subjects could reproduce the same control patterns regardless of perturbations. Test movements opposed by a high load ended near the peak velocity of control movements. Phasic and tonic EMG patterns were load-dependent. Results indicate that the control pattern is of short duration which suggests that rather than being pre-programmed, EMG signals represent long-lasting dynamic responses of the system to the short-duration control pattern, external forces and proprioceptive feedback.
SELECTIVE PROCESSES IN THE DEVELOPMENT OF REACHING. I. SINGLE ARM TRAJECTORIES. E. Thelen*, D. Corbetta, and J. P. Spencer. Dept. of Psychology, Indiana University, Bloomington IN 47406

Developmental studies provide a window on the neural mechanisms of hand trajectory control and reaching. It is well known that during the first year, human infants' hand trajectories become straighter, smoother, and more accurate. But reaches do not develop in isolation; rather, infants learn reaching movements in the context of coordinated variable non-reaching movements of the hands and arms. Here we describe reaching development as infants' discovery of stable trajectory parameters from a wider range of movement patterns.

We observed 4 infants (3 boys and 1 girl) weekly from 3 to 30 weeks and biweekly thereafter until 52 weeks as they reached for a toy at midline. Reaches were performed in 14 a trials, and hand trajectories were monitored by WATSMART motion analysis at 150 Hz before, during, and after the reach. Infants reached at 12, 15, 21 and 22 weeks and showed a dramatic improvement in trajectory straightness, smoothness and velocity modulation at 30, 32, 30, and 36 weeks, respectively. Prior to the transition, each infant had a long-month epoch of high velocity reaches, which in turn, disrupted the reach path stability. The speed increases were not unique to reaching, however, but were also characteristic of other non-reaching movements. After this active period, trajectory parameters stabilized.

Reaching improvement is not steady, linear and encapsulated. Rather, reaches are "cured out" of ongoing movements and are influenced by the ongoing movement context. Development consists of discovering consistent solutions after exploring a wide range of movement parameters. Supported by NIH R01 HD22830 and NIMH K05 MH01102

SELECTIVE PROCESSES IN THE DEVELOPMENT OF REACHING. II. INTERLIMB MOVEMENTS. D. Corbetta, E. Thelen. Department of Psychology, Indiana University, Bloomington IN 47406.

Early patterns of interlimb coordination associated with the development of reaching follow highly unstable and rapidly fluctuating forms over time. Reaching patterns alternate between periods of one- and two-handedness, and, during one-handed periods, no clear hand preference emerge. One interpretation is that developmental fluctuations represent the pre-organization of arm movements in the neuromotor system. Alternatively, we propose that developmental fluctuations emerge as "self-organized" properties of the system in interaction with the task.

We report the development of interlimb patterns over weeks, followed weekly from 26 weeks to 30 weeks and biweekly until 52 weeks as they reached for small toys at midline. Each week, we recorded the endpoint kinematics of both arms during multiple 14 asymptotic reaches using WATSMART motion analysis. The data we collected included both reaching and non-reaching activity. Specific movement analyses allowed us to capture the interlimb patterns of the reach itself and the ongoing activity from which the reach emerged.

We show that fluctuating patterns in reaching emerge from similar fluctuating patterns in the general non-reaching activity. We show that the general non-reaching responses modulate the movement over time and speed changes: when speed increases interlimb patterns become more synchronous and reaches more bimanual, while speed decreases, synchronous and bimanual patterns dissolve. We finally show that decreases in movement speed coincide with the emergence of interlimb asymmetries, revealing a shift toward an increased right arm activity. We argue that the emergence of lateral, one-handed reaching is progressively carved out of the general movement activity, through dynamic and selective processes.

Supported by NIH R01 HD22830 and NIMH K05 MH01102

TEMPORAL PARCELATION OF MOVEMENT KINEMATICS IN THE MUSCLE ACTIVITY OF MONKEYS. J.D. Cool*, Q.-G. Fu and J. J. Cash. Graduate Program in Neuroscience and Departments of Neurosurgery and Physiology, University of Minnesota Medical School, Minneapolis, MN 55455.

Previous electrophysiological studies of the motor cortices in our laboratory (Fu et al., 1993; Fu et al., 1994, in press) revealed timing differences in the encoding of movement parameters including direction, distance, and x-y position. Specifically, these parameters are encoded separately and sequentially, with direction-related discharge occurring first, x-y position-related discharge second, and distance-related discharge last. Our paradigm required monkeys to make reaching movements using horizontal plans, from a centrally located start position to 48 targets in 8 different directions and 6 distances. This study extends these observations to muscle activity during the same range of movements. Intramuscular EMG signals were collected in 15 muscles, and fitted to a multivariate time regression model. ANOVA and subsequent Tukey's Test were done on a subset of 14 muscles and revealed a clear temporal sequencing of parameter latency changes (p<0.001 by ANOVA)

Direction-related discharge occurred first (67±68 ms following movement onset), followed by x-y position-related discharge (254±151 ms), and distance-related discharge (497±165 ms). Latencies for each kinematic parameter were longer, and peak R2 values were smaller, than those for cortical cells. In addition, muscles were assigned according to their actions into upper arm, lower arm, and wrist/hand groups. Tukey's Test revealed no significant differences in discharge latency among the muscle groups for each parameter. These preliminary findings suggest that the temporal parcelation of movement parameters observed in the motor cortices is preserved at the level of muscle activation. Supported by NIH grants R01-NS18338 and R01-NS131530.

DIRECTIONAL PROPERTIES OF ARM MUSCLE ACTIVATION FOR DYNAMIC ISOMETRIC FORCES. J.T. Pollington* and M. Frander. Dept. of Physiology, Univ. Minnesota, Minneapolis, MN 55455.

We recently described how the timing and intensity of arm muscle electromyographic (EMG) activity vary with the direction of reach. After subtraction of postural activity, phasic EMG exhibited classic trisphasic burst patterns. The intensity of phasic activity was a multimodal function of movement direction, rather than a unimodal center-out function.

In the current study, we examined the patterns of activity in the same muscles during the discharging, as opposed to isometric forces. The surface EMG of 9 elbow and shoulder muscles was recorded while human subjects rapidly exerted 10N of force at the wrist in one of 20 directions in either a sagittal or frontal plane.

The onset of activity in a muscle varied with force direction: agonist bursts occurred in one range of directions, while antagonist bursts were generally occurred in the opposite range. As with reaching movements, the directional tuning of EMG intensity was multimodal. The frontal plane polar plots above illustrate that birefringence of intense EMG activity (greater distance from center) for ranges of directions including and in addition to straight up. Data are from one subject; left plot is from forces, right plot from 30 cm movements. Dynamic isometric forces will be useful for the study of the generation of complex motor patterns at the level of single motor units.


Previous work [1] on perceptuomotor coordination in houseflies has shown that learning a new behavioral pattern can be captured as specific alterations of the underlying coordination dynamics. The to-be-learned pattern (i.e., a frequency-locked pattern) is treated as a single modulation, and a phase-locked pattern is treated as a single modulation. For example, if a fly is trained to turn left, then left turns are increased. The dynamics of the learned behavior change from one that is random to one that is more predictable. The learned behavior is then repeated for a second pattern, and the dynamics of the learned behavior change from one that is predictable to one that is more random. The dynamics of the learned behavior are then repeated for a third pattern, and the dynamics of the learned behavior change from one that is random to one that is more predictable. The dynamics of the learned behavior change from one that is predictable to one that is more random. The dynamics of the learned behavior change from one that is random to one that is more predictable.

The current study investigates the dynamics of generalization of learning across two different coordination systems. Specifically, the dynamics of learning a new behavioral pattern are captured as specific alterations of the underlying coordination dynamics. The to-be-learned pattern (i.e., a frequency-locked pattern) is treated as a single modulation, and a phase-locked pattern is treated as a single modulation. For example, if a fly is trained to turn left, then left turns are increased. The dynamics of the learned behavior change from one that is random to one that is more predictable. The learned behavior is then repeated for a second pattern, and the dynamics of the learned behavior change from one that is predictable to one that is more random. The dynamics of the learned behavior are then repeated for a third pattern, and the dynamics of the learned behavior change from one that is random to one that is more predictable. The dynamics of the learned behavior change from one that is predictable to one that is more random.
408.18
LEFT LIMB MOTOR FUNCTION: THE BRAIN-DOWN BELT. W. A. Lee*, A. Murias, M. Clark, B. Macauley, L. Roth, K. M. Heiman, Center for Molecular and Behavioral Neuroscience, Rutgers University, New Jersey 07102; VA Medical Center and University of Florida, Gainesville, FL.

Many investigators have explored the differential roles of the left and right hemispheres in planning and executing skilled, learned movements, although few have analyzed the kinematic properties of these movements. It is suggested that the left hemisphere is critical for the control of skilled gestures but nonetheless, errors in movement performance have also been reported in subjects with right hemisphere damage. To further elucidate the contribution of the two hemispheres to skilled movement control, 3D motion analyses were performed on the trajectories of repetitive “slicing” gestures made by right unimpaired, left unilateral, and left hemispherical subjects. Four subjects with left hemisphere lesions and left apraxia, six subjects with right hemisphere lesions and seven neurologically intact subjects participated. Left-lateralized apraxic subjects, but not right-lateralized subjects, showed marked movement deficits. The apraxic subjects exhibited decoupling of the spatial aspects in the wrist trajectories and six additional navigational features. These deficits reflect the role of predictive and spatial aspects of the wrist trajectories.

408.19
PRODUCTION OF VOLUNTARY FINGER MOVEMENTS BY THE SUPPLEMENTARY MOTOR AREA: A STUDY OF THE PRIMARY MOTOR CORTEX.

Prominent direct projections from the supplementary motor area (SMA) to the spinal cord have been hypothesized to involve the production of direct movements of the primary motor cortex (M1) (Dum & Strick, 1991). We tested this hypothesis by determining the location and time course of intracortical activity time-locked to the generation of these movements by using two different approaches: (1) the normal analysis (DPA) and positron emission tomography (PET) simultaneously. Experiments were conducted to directly compare unilateral, repetitive finger opposition movements by using two different approaches: (1) DPA and PET simultaneously. In both groups, the effects of the SMA and the M1 were observed. PET data demonstrated that the SMAs involved significant activation of the contralesional M1 together with other structures in the parietal and intracortical pathways of the SMA. DSA and PET both showed that the SMAs were activated by activity in the SMA with minimal activation of M1. The SMA dipole orientation suggested that the SMAs were involved in neuronal populations in the dorsal tier of the cingulate sulcus. We hypothesize that the SMAs are involved via basal ganglia-thalamocortical pathways which do not include M1 and are separate from the pathways generating the SMAs. These SMAs are being investigated in subjects with Parkinson’s disease and neuroleptic induced Parkinsonism.

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408.20
SCHEMA LEARNING IN MULTIDIRECTION PULLING. W. A. Lee*, A. M. Russin, Y.-C. Pai and D. Schena. Prog. in Physical Therapy & Inst. for Neuroscience, Northwestern Univ. Med. School, Chicago, IL 60611 USA.

We studied whether perceptual-motor schema or exemplars are learned when subjects practice a multidirectional pulling task that involves movement of the whole body. Ten freestanding subjects practiced impulse-like pulls against a load cell to three force targets (20, 40 and 80% of maximum) on four days, receiving feedback with decreasing frequency. On a fifth day, subjects pulled to both the training and novel (10, 50, 60 and 95%) targets, receiving no feedback. On half of the trials each day, subjects were estimated the force produced while getting feedback. On Days 8 and 9 pulling sagittal plane body motion (11 markers) was recorded, from which force components associated with the location (Fgrav) and acceleration (Fcentrif) of the body’s center of mass were determined. Normalized actual and estimated force errors and similar Fgrav and Fcentrif proportions for training and novel pulls, while exemplar learning predicts larger errors for novel pulls.

Actual and estimated force errors decreased significantly with practice. Higher correlations between actual and estimated forces on Day 5 suggest parallel improvements in force perception and force learning. The actual and estimated training and novel pulls were comparable on Day 5, except for 10% pulls which had greater errors than all other pulls. The regression equations between force components and pulling forces were similar across conditions. These results support schema learning, but also suggest that much of the improvement may have been due to subjects’ learning to parameterize an already familiar kinetic schema for force production.

Supported by NSF IBN-9024186
Development of gonadotropin hormone releasing (GnRH) systems in the terminal nerve and brain of the cleared nose skate, Raja eglanteria, L.S. Dessauer, M. Barnes, and J. Snook. In: Carassius. Div. of Natural Sciences, New College of the Univ. of South Florida, Sarasota Fl, 34243.

GnRH-ir in both sexes was similar in older animals (16, 32 weeks and adult). The TN has well-developed ganglia of bipolar and multipolar cells that in the smaller fish span the length of olfactory bulb. The TN may enter the brain via dorsal and ventral roots. At 10 weeks, TN fascicles can be traced into the ventromedial telencephalon, from here bundles of ir-fibers extend into the septo- preoptic area and continue into the basal hypothalamus. Many fibers approach the infundibulum and portal vessels. A large ir- nucleus of bipolar and multipolar cells extends most of the length of the midbrain in all stages. In older animals, ir-beaded fibers are present in the neurohypophysis and most areas of the CNS; some may extend to the ventricular surfaces. A few scattered ir-fibers are present in the olfactory epithelium. Few ir-fibers are seen anywhere in the younger fish (7-8 weeks).


Sexually specific behaviors are frequently associated with sexually dimorphic brain areas. In particular, the ventromedial hypothalamic nucleus (VMH) has been associated with the female display of receptivity in lizards. We wished to determine if the VMH and two amygdaloid areas that project to the VMH, the lateral amygdala (LA) and medial amygdala, have sexually dimorphic volumes or cell sizes in a lizard, the Tokay gecko (Gekko gecko). Data was collected from 3 adult males and 3 adult females. Borders of the nuclei and somata were drawn on photographs of brain sections, then traced on a digitizing tablet linked with a computer to calculate areas and volumes. Only the values for the volume of the medial subdivision of the VMH were statistically significant, being larger in females than males. The values for the volumes of the lateral VMH and total VMH approached statistical significance with larger volumes in females. Measurements of somal areas revealed a marked sexual dimorphism, with females containing larger cells in the medial and lateral subdivisions of VMH and in the lateral amygdala. Somal areas of the other nucleus were not sexually dimorphic. Our data is consistent with previous findings in lizards and mammals that associated the VMH with sexual dimorphism and female receptivity. However, a sexually dimorphic telencephalic nucleus with larger somata in females has not previously been reported. The larger somal areas in the LA and VMH of female geckos and the projection from the LA to the VMH suggest that both the LA and VMH are involved in female-specific behaviors.


We used specific antibodies (Ab) recognizing the GluR1, GluR2/3, and GluR4 subunits of the AMPA receptor family (Chemicon) and the GluR5,6,7 subunits of the kainate receptor family (PharMingen) to localize these ionotropic glutamate receptor subunits in pigeon telencephalon. The GluR1 Ab labeled medium and large neurons that contained the densely labeled neuronal of dorsal and ventral striatum, while the neuropil and large projection neurons of dorsal and ventral pallium remained unlabeled. Scattered neurons in the prefrontal cortex were labeled for GluR1 throughout the pallium, while the palium ex. externum, archistriatum dorsale, nucleus taeniae, hippocampus, prehippocampal area, and hyperstriatum dorsale and ventrale stood out by their densely labeled neuropil. The GluR2/3 Ab was labeled in a wide variety of neurons in the prefrontal cortex, while the hippocampus and prehippocampal area of the palium. All palium regions possessed very abundant populations of GluR2/3 and GluR5,6,7, with highest levels of labeling found in the hippocampus and prehippocampal area. The distributions of these GluR subunits in the avian telencephalon share many similarities with those found in mammals, indicating similar importance for long-term transmitter in the avian pallium, and also for pallial and diencephalic inputs to the avian basal ganglia. Supported by NS-16620, NS-28721 (A.R.), & Neuroscience Center for Excellence (G.C.)


Early gonadal development in chicks can be induced via: 1) proaural hypothalamic knife-cuts, 2) chronic intraventricular injection of NPY, and 3) feeding sulfamethazine (SMX). An experiment was conducted to determine the neural effects of feeding 0.2% SMX to chicks beginning at one week of age. At 3 weeks, age 4 SMX-treated chicks and 4 controls were sacrificed, with saline followed by 4% paraformaldehyde and prepared for immunocytochemistry using antibodies to gonadotropin releasing hormone (GnRH) and NPY. A second experiment was conducted with 6 experiments, and 6 controls, perfused with saline followed by 4% paraformaldehyde and prepared for immunocytochemistry using antibodies to gonadotropin releasing hormone (GnRH) and NPY. An experiment was conducted to determine the neural effects of feeding 0.2% SMX to chicks beginning at one week of age. At 3 weeks, age 4 SMX-treated chicks and 4 controls were sacrificed, with saline followed by 4% paraformaldehyde and prepared for immunocytochemistry using antibodies to gonadotropin releasing hormone (GnRH) and NPY.


The saddleback wrasse Thalassoma duperrey is a sequential hermaphrodite that begins life as a subordinate female or male and may then transform into a terminal male via sex or role change. Terminal male fish aggressively dominate subordinates and may function to inhibit sex/role change. In fish, corticotropic-releasing hormone (CRH) and arginine vasotocin (AVT) are the primary hypothalamic secretagogues in the stress response. CRH and AVT cells in teleosts show a robust and overlapping distribution in the magnocellular and parvocellular preoptic area (POA). Immunocytochemical studies on the saddleback wrasse showed that the distribution of AVT cells reflects this general teleost pattern. Conversely, CRH did not conform to this pattern; in all but one individual, CRH cells were entirely absent from the POA. However, there were a unique group of CRH cells located in the Hypothalamus ventralis (HV). A comparative study using goldfish (Carassius auratus), a gonochoristic species conducted to determine these cells. Immunohistochemical analysis of the POA in CRH and AVT immunolabeling in the magnocellular and parvocellular POA indicated that 1) the goldfish is representative of the general teleost pattern, and 2) our results with T. duperrey represent a novel feature of a sex reversing fish. The paucity of CRH+ somata in the POA may be indicative of negative feedback from stress-induced elevation in cortisol, the unique group of cells found in Hv may play a role in stress-mediated control of sex reversal. The present results underscore the importance of these fish as a model system for investigations of neuroendocrine control of vertebrate sexual plasticity. Supported by NSF (IBN-9309565 to MGG and REU to MSG/AAG) and a University of Idaho Seed Grant.
409.7 IMMUNOHISTOCHEMICAL ORGANIZATION OF THE FOREBRAIN IN THE WHITE STURGEON, *P. fluviatilis* and R. G. Northcutt* Neurobiology Unit, Scripps Institution of Oceanography, and Dept. of Neurosciences, School of Medicine, University of California, La Jolla, CA 92030-2091.

To determine the palial and subpalial divisions in ray-finned fish, the distribution of leucine-enkephalin (LENK), substance P (SP), tyrosine hydroxylase (TH; dopamine) and dopamine-β-hydroxylase determined in the forebrain of an actinopterygian, Acipenser transmontanus (Chondrosti). Immunoreactive TH, SP, LENK, TH, and DAI penkarya were observed in different nuclei of the area centralis telencephali, pretectal area, periventricular nucleus of the posterior tuberculum (PPT), and inferior lobe of the hypothalamus in the ventral thalamus, few SVP+ and TH+ data are seen, whereas in the median nucleus of the posterior tuberculum only cells positive for TH were observed. A high number of SP+, LENK+ and TH+ fibers were seen in the nuclei of the area ventralis telencephali, whereas DA+ and SHT+ fibers were confined to the dorsal nucleus (Vd) of this area. All five substances were present in fibers innervating various diencephalic areas. Earlier studies suggested that the pallial-subpallial boundary was located between the medial (Dm) and the dorsal (Dd) zones of the dorsal telencephalon, and that Dm may be homologous to the striatum of land vertebrates. The present results do not support this view, since Dm showed poor content of SP+ and LENK+ fibers compared to the nuclei of the area ventralis telencephali. In this respect, Vd appears highly similar to the striatum, with a very high concentration of SP+, LENK+, TH, and DA+ fibers. This is supported by the presence of DA+ cells in PPT, a homologous region of the substantia nigra of amniotes, which could be the source of DApigcinput to Vd. Supported by NIH grant NS24869 to R.G.N.

409.9 A MARINE CHAMELEON: THE SANDLANCE LIMNICHYTHYES FASCATUS. J.D. Pettitross and S. P. Collins.* Vision, Touch and Hearing Research Centre, University of Queensland, St Lucia 4072 and Department of Psychology, University of Western Australia, Nedlands, 6009, Australia.

The sandlance, *Limnichthyes fascatus* (Creekfield, Teleosti), behaves like a marine chameleon, with independent movements of its turrets-like eye stalks that strike from camera-like, swimming prey. The optical system has a fixed circular pupil, a deep pit fovea and a flattened lens unlike any other teleost lens so far described. The cones, layered structure of the cornea is also unique, unparalledled in a teleost and suggests that the cones play a refractive role. This suggestion has been supported by four independent sets of observations: i) Photopic image formation can be observed by the eye; ii) Measurements of the magnification of intracorneal isodopes viewed through the corneal lenticle; iii) Measurements of the dissected corneal lens and lens when viewed under a microscope; iv) Ray tracing experiments comparing the degree of refraction produced by both the lens and corneal lenticle. All four sets of observations confirm that the cornea of the sandlance has a substantial refractive power of approximately 200 D compared with a lens power of 50 D. This is the first report of a teleost cornea with a significant refractive role. The optical system of lens plus cornea, in combination with the deep pit fovea, may be more suitable for the detection and accurate depth localisation of small, moving prey than the conventional, wide-field, spherical lens system of teleosts. The evolutionary convergence of this marine optical system and lifestyle with those of the chameleons is remarkable given the constraints imposed by underwater optics.


In amphibian and reptilian cell masses of the ventrolateral telencephalon are traditionally homologized to the striatum of amniotes. Comparative cytoarchitectural and topological studies of developing and/or adult forebrains of representatives of all three amphibian orders, together with topological and histochemical information about the striatum, suggest the following new hypothesis. The "striatum" of amphibians is pallial rather than subpallial and is homologous to the anterior ventricular ridge (ADV) of reptiles. This hypothesis is a more parsimonious interpretation of the data than the prevailing one, and suggests that the amphibian homolog of the amniote striatum may lie in the ventromedial telencephalic wall. It further suggests that the hypothalamic nuclei of all amniotic vertebrates may be subject to a similar reinterpretation. Taken together with the idea that the "striatal" ADV is homologous to the mammalian basolateral amygdala (Neary and Bruce, 1993), the possibility is raised that a thalamic-pallial-striatum amygdala is a significant and common feature of amniotic forebrains. (Support: NSF BNS-8620858 and Oberlin College)

409.12 CAVUM SEPTUM PELLUCIDUM (CSP) IN TREATMENT RESISTANT SCHIZOPHRENIA. R. Corone*, S. Davies, C. Goumans, C. Tamminga, R. C. Sanders. University of Maryland Psychiatric Research Center, Univ. of MD, Baltimore, MD 21228.

A number of investigations have focused on differences in clinical characteristics between people with schizophrenia who respond well to treatment and those who do not. Schizophrenics who fail to respond to clozapine may represent a separate population, with distinctly different etiology and/or neurobiology. Comparing treatment-responsive patients, one CT study found smaller ventricles in treatment-resistant patients (Ota et al., 1987), while another found greater prefrontal sulcal prominence in these patients (Friedman et al., 1991). Examination of the neuroanatomy in these two patient populations may provide further evidence to support a biological distinction between these. We examined MRI scans from treatment-resistant and treatment-responsive patients, and correlated the positions of the CSP, the third cerebral ventricle, and the lengths of the interventricular angle. Our results suggest that CSP in treatment-resistant patients is significantly higher compared to treatment-responsive patients. The CSP is significantly larger than the interventricular angle (12.5%), while the interventricular angle is significantly smaller than in treatment-responsive patients (11.9%). These data are consistent with the hypothesis that the CSP is a significant anatomic feature in patients with treatment-resistant schizophrenia.
HIGH RESOLUTION NMRI ATLAS OF AN INFANT MOUSE LEMUR (MICROCEBUS) BRAIN OBTAINED AT 12 T, Mark O'Dell*, Pratik Ghosh, Russell Jacobs, and John Aliman. Department of Computation & Neural Systems and Division of Neurology, Beckman Institute, Caltech 139-74, Pasadena, CA 91125.

In preparation for doing functional brain imaging, we have constructed a 3D registered digital atlas of the head of a deceased infant mouse Lemur (Primate M. Minimirus) using a Bruker AMX500 12 Tesla Nuclear Magnetic Resonance Imaging system (1H = 500 MHz). We obtained very high spatial resolution data (60 μm isotropic voxels) with strong intrinsic contrast (T2-weighted Spin Echo 3D), making it possible to see fine anatomical detail. We show in 3D volume renderings of this atlas on videotape, anatomical identifications on 2D prints, and details of data acquisition and processing.

Data was acquired using TE/TR = 100ms/1.3s with the sample at 2°C. While some MRI contrast is likely to be different from what would be obtained from a living specimen, all familiar anatomical structures are clearly visible. Fine fiber tracts, the laminations of corticis, and details of the inner ears are especially striking. Mouse Lemurs were selected because they have a highly developed visual system and are small enough to fit inside our imaging system.

BRAIN METABOLISM AND BLOOD FLOW: NITRIC OXIDE

1. CEREBRAL BLOOD FLOW (CBF), AND NEUROLOGIC OUTCOME IN RATS SUBJECT-ED TO TRANSIENT FOREHEAD ISCHEMIA: A COMPARISON BETWEEN CHRONIC HYPERCAPNIA AND SYNTHASE (NO) INHIBITION. V.L. Baughman, D.A. Pellicier*, and D. Wang, Dept. of Anesthesiology, Univ. of Illinois-Chicago, Chicago, IL 60612.

It is unclear whether the beneficial influence of NO during and following cerebral ischemia is protective (e.g., via promoting vasodilatation) or neurotoxic (e.g., through production of highly reactive O2-, NO, etc.). These opposing actions of NO could account for some of the variability in findings present in the literature. In this study, we subjected rats to 20 min of global cerebral ischemia and determined the effects of NO inhibition, with nitro-L-arginine (S-NA), on: 1) blood flow, 2) cerebral lactate accumulation, 3) neurologic function, and 4) histopathology postischemically. In order to differentiate the potentially protective vasodilating actions of NO from other (perhaps toxic) NO actions, 2 groups of L-NA-treated rats were examined: 1) a group receiving 10 mg/kg of L-NA i.p. 1 h before ischemia, which is the level of L-NA used to control (CT); 2) the other group (L-NA, n = 2) in which NO was not blocked for control purposes. In the L-NA group, aortic pressure was increased by 25-30 mm Hg postischemia. The anaesthesia was then discontinued. Rats were analyzed for neurologic function over 3 days. During ischemia, the CBF reductions measured were 65%, 89%, and 70% in the CT, L-NA, and L-NA2 groups, respectively. The corresponding MAP (mm Hg) values were 31, 31, and 52 mm Hg. L-NA treatment was accompanied by a greater post-ischemic hyperemic response than seen in CT (160%, 253%, and 193% of baseline in CT, L-NA, and L-NA2, respectively). Neurologic outcome in the L-NA group was markedly worse than CT. However, the mean score in the L-NA2 group suggested improved outcome vs CT, although the difference was not significant. These results suggest that NO is beneficial during TFI, presumably via an intra-ischemic vasodilating action. That benefit may be modestly opposed, but not negated, by a neurotoxic effect of NO. The greater post-ischemic CBF in the L-NA group indicates that, in this model, post-ischemcic hyperemia is not mediated by NO.


Chronic oral administration of the nitric oxide synthase inhibitor Nω-nitro-L-arginine-methyl-ester (L-NAME) results in increased hypothalamic vascular resistance (HVVR) (Benyo et al.; Society for Neurosciences, Abstacts, Vol. 19, 1990). The aim of the present study was to investigate whether this phenomenon can be reversed by L-arginine.

Male adult Wistar rats were treated with L-NAME dissolved in the drinking water. After a week of treatment, the animals were divided into 3 groups: control rats treated with ip. urethan, and hypothalamic blood flow (HBF, Hf/g clearances of mean arterial blood pressure (MAP) = 1304 ± 100 mmHg before the start of iv. L-arginine administration (300 mg/kg, bolus followed by 10 mg/kg/min infusion). Results: Control 10 min. 20 min. 30 min. 40 min. HBF (ml/min/g) 6.80±0.04 8.02±0.04 8.73±0.06 8.50±0.06 8.20±0.06 MAP (mmHg) 150±10 130±11 121±11 115±10 107±10 HVR (MAP/HBF) 22.5±21 162±19 151±20* 141±19* 134±16* apCO2 (mmHg) 35.2±0.7 36.3±0.9 36.5±0.9 35.3±1.1 34.1±0.9 apQ2 (mmHg) 83.6±2.4 84.0±1.4 84.1±1.8 86.8±1.7 88.6±1.2 apH 7.35±0.02 7.3±0.02 7.32±0.02 7.31±0.02 7.31±0.02 pCO2 = 0.05, *p<0.01 vs Control. ANOVA with Donnett "s test.

The results indicate that the hypothalamic vasconstrictor effect of nitric oxide inhibition induced by chronic oral L-NAME treatment can be reversed by an excess amount of exogenous L-arginine.

3. NITRIC OXIDE PLAYS DIFFERENT ROLES IN CEREBRAL METABOLISM AND VASULAR FUNCTION: EFFECTS OF HYPERCAPNIA ON SARAFON OR BASAL HYPERCAPNIA SIMULATION. F. Zanzoni* and C. Lapedeca, Dept. of Neurology, Univ. of Minnesota, Minneapolis, MN 55455.

Inhibition of nitric oxide (NO) synthase (NOS) attenuates the increases in cerebral blood flow (CBF) elicited by electrical stimulation of the basal forebrain (BF) cholineric system or hypercapnia. We sought to determine whether the effect of NOS on BF metabolism and CBF is mediated by alterations in NO levels in intrahippocampal- and ventilated rats the NO synthase inhibitors nitro-L-arginine (L-NAME) and carbonyl cyanide 3-chlorophenylhydroxamic acid (CCHPH) were superfused by a laser-Doppler probe. Inhibiting NO activity was verified using the assay of Brett and Snyder. With ringer solution, BF stimulation (0.10-0.56 Hz) increases CBF by 214±18% (n=5) and 130±11% (n=6). However, the cerebral blood flow increases were attenuated by L-NAME and 3-chlorophenylhydrazine-1-carboxylic acid (L-NAME) sodium acetate (45±4%; p<0.001). Superfusion with CBF by L-NAME reduced CBF by 33±4% (p<0.05) attenuated the increase in CBF generation by l-arginine (L-NAME) acetate or by hypercapnia (45±4%; p<0.001) and reduced NO synthase catalytic activity by 93±3%. After L-NAME superfusion with the NO donor SIN 1 (0.1-0.5 HZ) re-established resting CBF (p<0.05). L-NAME superfusion by NO donor failed to increase CBF response to hypercapnia (p>0.05 from before L-NAME). However, SIN 1 failed to counteract the L-NAME-induced attenuation of the increase in CBF elicited by stimulation (p<0.05 from before L-NAME). We conclude that both the CBF responses to hypercapnia and BF stimulation have NO-dependent components. However, the role of NO in these responses differs: In hypercapnia, unlike BF stimulation, the NO-dependent component can be fully re-established by a NO donor despite near total inhibition of NO synthase catalytic activity. The data suggest that the CBF response evoked by CBF stimulation requires NO synthesis, probably via cortical release of acetylcholine, whereas the hypercapnic response inactivation requires a basal level of NO for its full expression. (Supported by NS 31318 and the AHA)

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NITRIC OXIDE SYNTHESE IS REQUIRED FOR FUNCTIONAL HYPEREMIA IN CEREBELLAR CORTEX. L. L., X. Xu and C. Iedecote, Dept. of Neurology, Univ. of Nebraska, Lincoln, NE.

Functional brain activation is associated with increases in cerebral blood flow (CBF) restricted to the activated region. Although the mediators of the vascular response remain to be elucidated in full, there is evidence that nitric oxide (NO), a potent vasodilator released by active neurons, may participate. We used the parallel barometric (PB) system of the cerebral cortex as a model to investigate the role of NO in the increases in CBF elicited by neural activation. Rats were anesthetized with halothane and ventilated. The cerebellar vermis was surgically exposed and maintained in situ throughout the experiment. CBF was measured using silver microelectrodes. During Ringer superfusion (n=7), CBF increased by an average magnitude of 33±9% (p=0.01) and the increases in CBFs were reduced by 50±10% (n=7; p=0.01; analysis of variance) and inhibited NO catalytic activity, as detected by L-arginine to citrulline conversion, by 95±9% (p=0.01). L-NAME did not influence the increase in CBF, but did reduce the response by 41±9% (p=0.01). Thus, the increases in CBFs were evoked by NO scavenger methylene blue (1 mM) and were reduced by the presence of L-NAME, but did not affect the response (p=0.05). The gait instability and NO scavenger methylene blue (1 mM) were reduced by the presence of L-NAME, but did not affect the response (p=0.05). The gait stability was assessed by the presence of L-NAME, but did not affect the response (p=0.05). The gait instability was reduced by the presence of L-NAME, but did not affect the response (p=0.05). The gait instability was assessed by the presence of L-NAME, but did not affect the response (p=0.05).

(Supported by NS 31318 and by the American Heart Association).

410.7

THE EFFECT OF NITRIC OXIDE SYNTHASE INHIBITION ON CAROTID OUTPUT AND REGIONAL ORGAN BLOOD FLOW IN PIGLETS. Lehto S.A., R. Prasad, R. Schilling, and D.M. Panum, Dept. of Pediatrics, University of Michigan School of Medicine, Miami, FL 33101.

We examined the effects of low dose N-nitro-L-arginine methylester HCl (L-NAME) on cerebral and regional blood flow in piglets. Although there was no change in regional cerebral blood flow, there was a significant decrease in cardiac output. Piglets were anesthetized with ketamine and xylazine before the administration of L-NAME (0.5 mg/kg, i.v.). Cardiac output and regional blood flow were measured using electromagnetic flowmeters placed on the main arteries and veins, respectively, after cannulation of the femoral artery and vein. After 30 min of L-NAME, cardiac output was reduced by 25±5% (p<0.05) and all regional vascular beds showed a significant decrease in blood flow (all p<0.05). The decrease in cardiac output was due to a decrease in myocardial contractility but not a reduction in cardiac output after L-NAME administration, without a significant change in MAP.

410.8

NITRIC OXIDE SYNTHASE (NOS)-CONTAINING NEURONS INNERVATE HUMAN CEREBRAL MICROVESSELS. W. Li, Z. Wang,1 D. Liu, M. Lopez-Fernandez,2 and K. H. Oldendorf,3 Dept. of Surgery, Univ. of Tenn, M.D. Hosp., and Panum Inst., The Univ. of Copenhagen, Denmark.

Nitric Oxide (NO) is a potent vasculor and neuronal messenger molecule generated from L-arginine by NO synthase. Although data indicate that NO is important in the modulation of human cerebral blood vessel relaxation, there is little information about the localization of NOS in the human cerebrovascular bed. In the present study, we have used NADPH-diaphorase (NOS)-induced histochemistry to examine the existence and distribution of cerebrovascular neurons containing NOS. Four blocks of human temporal parietal cortex were cut on a vibratome at 50-100 mm sections. Two of five series were reserved for NADPH-diaphorase staining. While two of five series was prepared for immunocytochemistry for NOS reaction by a monoclonal antibody against the neuronal isoform of the enzyme and the remaining was used for an immunohistochemical demonstration of neurons with NADH diaphorase activity. We found that NADPH-d and NOS positive staining was located in cells and nerve fibrillae surrounding cerebral vessels. Differential NADPH-d and NOS specific and nerve fiber staining was noted at various sites of the cerebrovascular bed. In some cases, NADPH-d and NOS positive neurons in the cortex were seen close to and sending processes to the neighboring vessels; in other cases, the cell bodies were located far from the microvessels, sending processes to the blood vessels. NADPH-d and NOS specific positive fibrillae were also situated on the wall of blood vessels and had processes that paralleled with longitudinal direction of the vessels. Part of the vessel wall with stained fibers contained varicosities. Cell bodies were oval, fusiform and triangular with one to three primary dendrites of variable length. Some of primary dendrites by second dendrites. The identity of neurons and fibers containing NOS in human microvessels provides morphological evidence for a role of NO in modulating human cerebral blood flow.

410.9

THE EFFECT OF 7-NITROINDAZOLOXIDE, AN INHIBITOR OF BRAIN NITRIC OXIDE SYNTHASE (NOS), ON BASAL CORTICAL BLOOD FLOW AND ON VASODILATION INDUCED BY WISKER STIMULATION. J. Zawadzki,1 G. Santanu,2 M. McGuigan,2 and A. St John3, Department of Anatomy & Neurobiology, University of Tennessee, Memphis, TN 38163, Universita di Roma "Tor Vergata", Rome, Italy. 1.

The brain vascular system is regulated by nitric oxide (NO) produced by endothelial and neuronal NO synthases. While the vasodilatory action of endothelially produced NO is well documented, the role of neurally derived NO in cerebral circulation is still uncertain. Recently, an apparent NO donor inactivates NO in the somatosensory cortex associated with neuronal activation elicited by vibrational stimulation. Sprague-Dawley rats were anesthetized with urethane and a-chloralose. Body temperature, blood gases and blood pressure were monitored. One of the cortical NOS inhibitors was measured by a Laser Doppler flowmeter probe. Barre cortex was activated by manual deflection of the contralateral vibrators for 15-30 seconds. There were no significant changes in basal CBF or in dilatory responses induced by vibrational stimulation following vehicle (i.p.) injection. Basal CBF decreased significantly (p<0.05) in 30 minutes after 7NiN I injection (i.p. in 3 ml of peanut oil) with minimal reduction of 7NiN I (n=8) and 59% (n=3) of pre 7NiN baseline at doses of 50 mg/kg and 75 mg/kg, respectively. A slight (10-12 mg/kg) (p<0.05) blood pressure was observed after 7NiN I injection. The magnitude of the dilatory responses were decreased after 7NiN I injection at 50 mg/kg (88%) and 75 mg/kg (43%), but the reductions were not significant (the latter case probably due to the small number of animals yet tested). These studies revealed that 7NiN I reduces basal cerebral blood flow presumably via inhibition of neuronal NOS and has a slight vasopressor effect. Les NOS inhibition is required to affect basal cerebrovascular tone than to affect dilatory responses associated with neuronal activation.

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410.10

VASODILATORY ACTIONS OF CALCITONIN GENE-RELATED PEPTIDE AND NITRIC OXIDE IN PARENCYHMAL MICROVESSELS OF THE RAT HIPPOCAMPUS. A. Ferguson, Y. Jin, Q.A. Thai, N.F. Kessell, K.S. Lea, Dept. of Neurological Surgery and The Neuroscience Graduate Program, Univ. of Virginia, Charlottesville, VA 22908.

Calcitonin gene-related peptide (CGRP) and nitric oxide (NO) are known to exert vasodilatory actions in a variety of vascular beds. Recent evidence suggests that CGRP and NO may mediate some aspects of the vasodilation elicited by NO. The present studies examined the responses of parenchymal microvessels in the rat hippocampus to CGRP and an inhibitor of nitric oxide synthase. Hippocampal slices were prepared from adult male Sprague-Dawley rats. Microvessels in the neuropil of submersed slices were examined using computer-assisted videomicroscopy. Drugs were administered by addition to the medium superfusing the slices. The resting diameter of vessels analyzed in this study ranged from 12 to 25 micrometers. Treatment with the nitric oxide synthase inhibitor, N-nitro-L-arginine (NNA; 100 μM), constricted vessels to 51.1±7.7% of resting diameter (n=9). Application of CGRP (10 μM) in the presence of NNA resulted in the dilation of the preconstricted vessels to 97.0±6.4% of their resting diameter (n=4). Three findings suggest that NO participates in the regulation of microvascular function by providing a tonic dilator influence. The ability of CGRP to dilate vessels in the presence of NNA suggests that CGRP-induced dilation is not dependent on production of NO. Ongoing experiments are investigating the possible role of CGRP in mediating NO-induced vasodilation.
411.1
MISMATCH-NEGATIVITY IN DIFFERENT BRAIN STRUCTURES - A TOPOGRAPHICAL AND DIMENSIONAL STUDY. M. Molnar, V. Czep, J. Wieder, J.B. Skinner and G. Kemeny. Institute for Psychology, Hungarian Academy of Sciences, Budapest, Hungary; Totta Gp Medical Research Laboratories, Bangor, USA. The purpose of the present investigation was to test: 1) if in animal experiments an attempt was made to record the mismatch-negative (MMN) in other brain structures than the auditory cortex, the latter being the hypothetical generator for the MMN. In chronically implanted freely moving cats auditory evoked potentials were recorded from the auditory and association cortices, dorsal hippocampus, amygdala, dorsal raphe nucleus and vertex. Auditory stimuli were short tones of 4 kHz frequency ("standard") randomly interspersed by 2 kHz "deviant" tones (probability : 1/10). 2) In human studies the mathematical tools of non-linear dynamics was used to analyze data collected in MMN-experiments from different scalp locations. The MMN, elicited by random stimuli within series of 1000 Hz deviant stimuli (probability : 1/10). The results suggest that, in contrast to its action in primary microvascular beds, does not affect the EEG and endo-endothelium interactions in the peripheral microcirculation. Supported by the DFG and the Wilhelm Sander Stiftung.

411.2
COMPARATIVE ANALYSIS OF CORTICAL GENERATORS OF MISMATCH-NEGATIVITY IN THE CAT AND MONKEY. G. Kemeny, J. Ulbert, D.C. Javitz, M. Molnar, V. Czep, Zs. Pirnitz and G.E. Schroeder. Inst. for Psychology of the Hungarian Academy of Sciences, Budapest, Hungary, Dep. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461. Human source localization techniques was implemented an investigation on the source of the mismatch-negative (MMN), which is a negative component of the scalp recorded auditory evoked potential (AEP). It is elicited by infrequent, 'deviant' stimuli, presented randomly within sequences of repetitive "standard" auditory stimuli. MMN appeared also in cats and monkeys as a negative component in the epidural AEPs in the latency range of 40-120 ms. The half area under the MMN generator the fields potentials and multiple unit activity were recorded from the auditory areas of awake cats and macaque monkeys by intracortical multielectrodes. One dimensional current source density (CSD) analysis was used to localize intracortical ictal sources. Surface maps in cats indicated the spread of the frequency MMN to both AI and AIJ auditory areas. In the middle layers of the auditory cortex of cats large amplitude local positive field was elicited by the standards in the latency range of the MMN while this positivity appeared with much smaller amplitude to the deviants. CSD analysis and the increased unit response elicited by the deviant stimulii suggested that the MMN represent a local disinhibition in the upper layers. In the AI area monkeys surface negative MMN to deviant stimuli was accompanied by an increased laminar II/III sink.

The data indicate that the MMN generators are localized in the supragranular layers of the auditory cortex. Supported by Oskta Grant 1/2595 and by the J.S. McDonnell Foundation.

411.3
THE GENERATOR QUESTION: A COMPARISON OF EVENT RELATED POTENTIAL (ERP) PROFILES WITH RECONSTRUCTIONS FROM CURRENT SOURCE DENSITY (CSD) ANALYSES. G. Czep, G.E. Schroeder, Dept. Biopsychology, NYS. Psychiatric Institute, NY/NY and Deps. Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461. One-dimensional current source density (CSD) analysis dealt the temporal and spatial patterns of transmembrane current flow which underlie the local field potential, and allow inferences about the likelihood that the sampled activity is a recording a distance. In contrast, image models (e.g. dipole models) simplify a scalp topography by indicating locations and waveforms for putative generators based on statistical and geometric inferences. A synthesis of these two approaches would provide a distinct advantage for the study of ERP generators. We recorded the electroencephalograms in the monkey while allowing the functional nucleus (LGN) and reconstructed the ERP profile from computed CSD profiles. CSD profiles were computed at low or high spatial resolution to bias the profiles toward open or closed field activity, respectively (Teske et al., 1993). Weighting functions for the cortical tissue and underlying distributed cylindrical generators (Nicholozon and Linna,1971). Reconstructed and empirical profiles were compared and residual waveforms computed from their differences. Low resolution methods produced a spectral roll-off better overall fit to the empirical profile. High resolution methods produced an oscillatory residual, reflecting closed field activity. Factors common to empirical and reconstructed profiles were extracted using a principal components analysis, to summarize them and to provide a direct linkage between local generators and the ERP recorded within and superficial to LGN. This approach shows promise for computing waveforms of volume conducting, regional dipoles directly from measurable properties of a region.

The electric currents in brain areas which are active during somotorimotor and cognitive processes produce electric and magnetic fields outside the skull which can be measured in real-time as event-related electric potentials (ERPs) and magnetic fields (ERFs) respectively. The accuracy of localizing active brain sources may be improved by combining ERP and ERF data since they are complementary. Prior to recent technological advances, it has not been possible to simultaneously record both electric and magnetic field data over the whole scalp in a single experimental session. We report here, simultaneously recorded electric and magnetic responses to visual stimuli with large sensor arrays (122 magnetometers and 32 electrodes) over the whole scalp. A series of retinotopic, pattern discrimination and attention experiments were conducted. The retinotopic results are presented here. ERPs and ERFs were recorded to small checkerboard wedge stimuli delivered to each quadrant. Source configurations for the initial responses were estimated from spatotemporal dipole analyses and minimum norm estimation. The locations of ERP and ERF convergence, and followed the expected retinotopic pattern for striate cortex, i.e., upper, right, lower, right, lower left and upper left quadrant stimulation activated lower left, upper left, upper right and lower right striate cortices respectively.

411.6 VALIDATION STUDIES OF A PROBABILISTIC SOLUTION TO THE EEG INVERSE PROBLEM - D.M. Price¹, F. Pigeot¹, M. Singh¹, Andrea Gerontolgy Center¹ and Dept. of Biomedical Engineering¹, Unv. of Southern California, Los Angeles, CA 90089-0123. The EEG inverse problem is to reconstruct the distribution of currents within the brain which generate the scalp measured potential distribution. This problem has an infinite number of exact solutions, so a probabilistic solution would seem to be necessary to solve this problem. The approach employed here maximizes the entropy of the source distribution subject to a system of linear equations which require that this distribution fits the scalp measured potential distribution. As is well known, the joint probability of sources in opposite directions at the same location is zero. With this formulation, this maximum entropy solution is the probability distribution for the source(s) of the measured potential field, and it is also the most likely source distribution for the measured potential field. In addition, the current probability distribution can be easily derived from this source probability distribution. The present studies were to provide initial validation for this approach. With simulated potential fields produced by a number of equivalent current dipoles in homogeneous sphere model, the current probability distributions approximated the actual equivalent current dipoles which generated the potential field. With a potential field measured at the human scalp during visual checkerboard stimulation which straddled at 3 Hz, the current probability distribution computed through a four sphere model was largely concentrated in the posterior regions of the brain including a region roughly corresponding to the primary visual cortex.

411.7 TOMOGRAPHIC MAPPING OF TEMPORAL LOBE P300 ACTIVITY IN THIN-DIMENSIONAL: ASSESSMENT OF RELIABILITY AND ACCURACY - J.C. Kastner¹, J.R. Price¹, J.A. Drouhard², C.的观点 University of California, Los Angeles, CA 90089-0123. A noninvasive interelectrode computer graphics algorithm for imaging the distribution of the P300 event-related potential within the temporal lobe is described. This technique takes advantage of the fact that the primate temporal lobe may be noninvasively surrounded by 4 electrodes forming a tetrahedral array consisting of three surface leads (CA, YA, and TA) and one nasopharyngeal lead (PG2). It is then possible to calculate the potential distribution within the enclosed volume of the temporal lobe given that the field strength Ep for any point p within the tetrahedron may be represented by the sum of the field contributions to or from the surrounding vertices, weighted by the distance between point p and each of the vertices. As part of an effort to assess the reliability and accuracy of this technique, monopolar recordings were obtained from the tetrahedral array in each of several squirrel monkeys (Gallium sciuereus) using a passive oddball paradigm. Simultaneous monopolar recordings were made at indwelling electrodes from a region of depth of 10mm to a final depth of 22mm within the temporal lobe, and these measurements were used to adjust the imaging algorithm to account for variations in head shape and conductivity via a least-squares technique. When applied to other subjects in a cross-validation procedure, the adjusted algorithm was found to yield accurate and reliable measurements at varied depths which were highly and significantly correlated with simultaneous measurements obtained from indwelling electrodes at corresponding sites.

411.9 BRAIN ACTIVATION WITH A MAZE TEST: AN EEG COHERENCE ANALYSIS STUDY IN HEALTHY SUBJECTS - M. Tremblay, D. Larose², V. Fraile, Y. Chaput², R. Lemier and J.-M. Abi-Rached, Service de Psychiatrie, Hôpital Notre-Dame, Université de Montréal, 1520 Sherbrooke E., Montréal, CANADA H2L 4M1.

The maze test is a complex cognitive task involving visuo-perceptual abilities and executive functions, such as planning and foresight. EEG coherence analysis is performed on the electrical signal of two electrodes and its value is analogous to a correlation coefficient between the two EEGs. Two recordings were done from 20 healthy subjects (mean age ± S.D.: 27.4 ± 4.23) during a maze test (3 mazes from the WISC and 2 from the Porteus) and during two baseline conditions: 1- rest with eyes opened; 2- mazes with dotted lines showing the way out. 19 electrodes were used according to the 10/20 system. Signals were referenced to averaged signals from both ears lobes (TO 3a, Filter 36 Hz). Interglutional coherence (RC) refers to coherence between each electrode pair to the remaining electrodes and may reflect dynamic physiological processes between brain regions during cognitive activation. Statistical probability was evaluated using the nodal regional distribution of changes. The results show that: 1- compared to the eyes opened condition, the maze test provoked increases of RC mainly located between paired posterior electrodes (mostly parietal P3 and P4 sites) and decreases between all fronto-frontal sites; 2- compared to RC of the 2nd baseline condition, the maze test provoked increases of RC mainly between paired posterior electrodes in 9 and 1/2 bands and between the left prefrontal F3 site paired with frontal or anterior sites in β band and provoked decreases between centro-parietal sites paired with frontal sites in γ. EEG coherence analysis detects electrical changes in regions known to be involved in visual-analytic, visuo- and executive functions (posterior, central and frontal sites respectively).


Event-related brain potentials (ERPs) were recorded during a syntactic decision task. Twenty-five right-handed young adults were presented with a total of 198 eight-word sentences, that were divided in three different groups: 66 Pseudo-Cleft Agent, (LO QUE UN ELEFANTE EMUEVO UN OSSO, "WHAT AN ELEPHANT PUSHED WAS A BEAR?"); 66 Pseudocleft Passive (UN ELEFANTE FUE LO QUE EMUEVO UN OSSO, "AN ELEPHANT WAS WHAT PUSHED WHAT?")) 66 sentences which were presented randomly intermixed. Subjects had to select the agent in each sentence. Scalp electrical activity was recorded from 32 derivations of the 10/20 international system. ERPs were registered to the seventh and eight word. Peak amplitudes were visually identified by windows of 200 msec each one (from 50 to 900 msec). ERP measures associated with both groups were subjected to ANOVA and repeated measures. Wave differences between ERPs were obtained. A slow negative response between 300-500 msec largest over anterior regions of the left hemisphere. These data reflected an negative component around 500 msc elicited by syntactic decision.
411.11 APPEARANCE OF FRONTO-PARITAL MIDLINE (FPm) THETA ACTIVITY IN GOOD AND POOR LEARNERS
S. Jakubka, J. Lindqvist, A. Bryilehto, Laboratory of Developmental Neuropsychology, University of Oulu, Linnunlahti P.O. BOX 222, FI-90901 Oulu, Finland.

Fronto-parietal theta activity (FPm, 4-7Hz) was investigated during learning process of problem solving in a simulation traffic situation on the subject had to learn to find the right way for driving a car through a set of roads by trial and error. Two independent driving situations had to be made at two crossroads guided by two traffic signs. After learning the level of performance was tested in a practising situation. Feedback about quality of performance was given. EEG was recorded from Fz and Pz. In learning situation, the amount of appearance of theta activity did not differ either on the frontal or on the parietal area between good learners and the poor learners. In the practising situation, poor learners showed a significantly higher amount of theta than good learners in the frontal and parietal area.

The results demonstrate a relation between the appearance of fronto-parietal midline theta activity and the quality of performance.


This study examined the effects of a cue-directing stimulus and the length of the cue-target interstimulus interval (ISI) on (1) stimulus-elicited brain responses occurring prior to and following the occurrence of relevant (validly cued) and irrelevant (invalidly cued) target stimuli in a given visual field; and (2) on latency (RTs) and accuracy (d-primes) of responses to cued stimuli. On a given trial a foylely presented arrow cued the subject to attend (without moving the eyes) to either the right or left visual field. After a brief delay a target appeared randomly either in the target stimulus position or in a position 154, possibly leading to a mismatch. Significant differences were found between the two ISI conditions.

411.13 CORTICAL EVOKED POTENTIALS DURING A VISUAL CONTINUOUS PERFORMANCE TASK. A. Teko-Kilco, E. Benders, D.W. Shupert*, Dept. of Neurology, SUNY @ Buffalo, 100 High St. (6E), Buffalo, NY 14203.

The Continuous Performance Task (CPT) was originally developed for the behavioral assessment of sustained attention in brain damaged patients (Rosvold et al., 1956). Different types of CPT's have been used to investigate attention in psychiatric and neurologic patient groups. It has become important to control for possible cognitive factors measured by the CPT in normal subjects. One of the variations of the CPT is the visual "A-X" paradigm (Hapler et al., 1988) in which subjects are asked to respond as fast as possible to the target letter "X" only if it is preceded by "A". Non-target letters preceded by "A" and "X" not preceded by "A" (non-target "X") are also presented. In order to study the physiological aspects of sustained attention, we examined the relationship between a cortical evoked potential component (P300) and the "A-X" paradigm. P300 is an endogenous component that is elicited by stimuli that are relevant to the subject. We expected that the P300 component would have the highest amplitude for both target "X"s and for other letters immediately preceded by "A". Eleven subjects were usually presented as stimuli in a random sequence of 400 letters, with 40 "A-X" pairs (p=0.10) and 68 "A-X" pairs (p=0.17), stimulus duration was 200 msec and ISI was 1500 msecs. Electroencephalographic activity was recorded at 12 scalp electrode sites of the 10-20 system, referenced to linked earlobes.

As reported in previous studies, the P300 component was most prevalent in the midline scalp sites. As predicted, the highest amplitude responses were observed for both target "X"s and other letters that were preceded by "A"s. These results are consistent with findings showing that task relevance is an important antecedent condition of P300 amplitude. The electrophysiological data provide information not attainable through behavioral observation about possible cognitive events underlying CPT performance.

411.14 THE EFFECTS OF PRECEDING INTERSTIMULUS INTERVAL ON BEHAVIORAL DISCRIMINATION AND MISMATCH NEGATIVITY.
M.G. Woldorf* and M. Matsuji, Univ. of Texas Health Science Center, Research Imaging Center, San Antonio, TX 78284-6200.

The mismatch negativity (MMN) is elicited by infrequent, physically deviant sounds in a sequence of repetitive auditory stimulus. It has been shown that this wave reflects a strongly automatic feature-analysis and mismatch-detection process, in which each stimulus is compared to the sensory template formed by the repeated standard-tone stimulus. This framework asserts that the MMN is largest at shorter interstimulus intervals (ISIs), due to the comparison template formed by the previous standard and being stronger. For similar reasons, this framework also predicts discriminability of the deviant stimuli should be better at shorter ISIs.

To explore this relationship more directly, subjects were monaurally presented with tone pips at ISIs ranging randomly from 125-925 msec, with 10% of the tones either slightly faster in intensity or slightly lower in pitch (different blocks). On half of the runs, subject tried to discriminate the deviant stimulus; on the other half, subjects were asked to count the number of tones. Pairs of tones were divided into 4 subranges, and target discrimination and ERPs were subdivided as a function of the ISI subrange of the preceding standard tone. The discriminability of the deviant target tones was strongly affected by preceeding ISIs, with subjects doing progressively worse at the shorter ISIs. Preliminary ERP results suggest that the reading-condition MMNs and the active condition MMN/DRN (deviance-related/negativity) also decreases with shorter ISIs. These data do not fit into a framework of increasing standard-tone template strengthening with decreasing ISI. Rather, as suggested by Woldorf and Hillyard (1991), a type of neural refractoriness appears to play a major role in these phenomena at shorter ISIs.

411.15 VEP ESTIMATION OF INTERHEMISPHERIC RELAY TIME.

Lines, Rugg & Milner (1984) dissociated a non-sensory from a sensory component of interhemispheric relaying (IR) by showing that the asymmetry in N160 latency of visual evoked potentials (VEP) to contralateral targets was abolished in bilateral stimulation in the occipital area only at occipital sites (about 11 and 18 msec for bright and dim stimuli), not at central sites (about 3 msecs). It appears possible, however, that the late 3 msecs delay simply represents different shifts in the apparent peak latency of a synchronous symmetrical source, due to superimposed asymmetrical potentials of occipital origin, the central N160 peaking during the rising phase of the occipital N160. To allow for more detailed topographic analysis, we obtained VEP from 21 scalp sites in 10 subjects in a simple reaction time task to yellow squares on a blue background, about 10° left of field of view. Preliminary N160 latency analysis shows a strong antero-posterior gradient, from 154 msecs at F3/F4 to 190 msecs at O1/O2. The IR estimates a parallel gradient from 5.9 msecs at O1/O2, with a value of 10.3 msecs at C3/C4. The latter, with a standard error of the mean of about 3.2, is a significantly larger asymmetry than the results earlier at C3/C4. The notion of simply two levels of IR in VEP appears to be an oversimplification.

411.16 ACTIVATION OF DURATION-SENSITIVE AUDITORY NEURONS IN HUMANS.
C. Alain*, D.L. Woods, and D. Covarrubias. Department of Neurology and Center for Neuroscience, UC Davis, Northern California System of Clinics, Martinez, CA 94553.

Auditory neurons vary in their duration tuning: some neurons fire to tones of short duration, whereas others require longer duration tones. In the current study, auditory evoked potentials (AEPs) were elicited by stimuli varying in duration (8, 24, or 72 ms) and frequency (250, 1000, or 4000 Hz) in an effort to characterize temporal integration functions (amplitude/latency changes with changes in tone duration) for different AEP components. High frequency tones used with all three durations were delivered randomly to the left and right ears. Subjects attended to a designated location and frequency to detect occasional long- or short-duration targets. Subjects were more accurate and faster at detection of long- than short-duration targets. Parallelism, this AEP components increased in amplitude and decreased in latencies with increased signal duration. Since all signals had identical rise-fall times and peak-to-peak amplitudes, the increase in latency difference waves could be isolated by subtracting AEPs to short-duration tones from AEPs to longer duration tones at the same location and frequency. Duration difference waves varied in amplitude and scalp distribution for tones of different frequencies. These different integration time for different frequencies and generators, with nonotopically organized AEP generators generally having longer temporal integration times.

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411.17 EVENT-RELATED DIFFERENCES IN EEG SPECTRAL POWER (ERDISP) D. L. Woods*, C. Alain, D. Covington, and M. P. Bailer, Dept. of Neuroscience and Neurosciences Center, UC Davis, Northern California System of Clinics, Martinez, CA 94553.

Recent studies using narrow band filtering techniques have shown changes in alpha (8-13 Hz) and gamma (30-50 Hz) frequency bands of the EEG in association with the sensory and cognitive analysis of signals. A simple model of discrimination was designed to examine Event-Related Differences in EEG Spectral Power (ERDISP) over the entire EEG frequency spectrum. Power spectra from brief EEG epochs preceding stimulus delivery are subtracted from power spectra following the stimulus. The selection of the epoch duration is critical to a trade-off between temporal and frequency resolution: short EEG epochs provide good temporal resolution but poor frequency resolution, whereas long EEG epochs provide good frequency resolution but poor temporal resolution. An analysis strategy using multiple epoch durations is proposed. ERDISPs reflect both sensory and cognitive processes. ERDISPs relating specifically to higher cognitive processes were calculated in ERDISPs using multiple subtractions. For example, for the effects of selective attention on ongoing EEG rhythms can be analyzed by subtracting ERDISPs from nonattended stimuli to ERDISPs to the same stimuli when attended. Δ-ERDISPs in selective attention tasks show alterations in several EEG frequency bands. The relationship between ERDISPs and event-related brain potentials (ERPs) and between ERDISPs and other techniques for analyzing event-related changes in EEG power will be discussed. Supported by NS38933 and DC10049.

411.19 SPECTRO-TEMPORAL CORRELATIONS IN HUMAN GAMMA BAND ELECTROCOGGRAMS. Y.硕士学位, W. W. Harms, and S. A. Graham, UCLA Dep't of Physiology, Los Angeles, CA 90095 and Division of Neurology, University of California, Berkeley, CA 94704.

Animal electrocorticogram (ECoG) studies have shown that coherent spatial patterns in the gamma band (> 20Hz) effect perceptual categorization. Spectro-temporal correlations were investigated in the 20-50Hz range in search for similar phenomena in human ECoG. The ECoG was recorded in a somatosensory discrimination task from a 64-electrode subdural grid array with spacing of 1cm between electrodes, overlaying somatosensory, motor, and supratemporal cortex. In two patients with intractable epilepsy. Stimulus and response related modulations of the μ, β, and γ bands of the 1/f spectrum were observed over intervals of 100ms in the somatosensory and motor cortices but not in the temporal cortex. No evidence was found for globally coherent responses related to behavior in the narrow (e.g. 40 Hz) or broad bands. Instead, spatially and temporally intermodulated synchronization was observed between pairs of electrodes with high variability within and across trials. The distribution of correlation coefficients differed substantially from those observed in the monkey cortex. The findings suggest that domains of spatial coherence in human gamma band ECoG are limited to < 2cm, that the intermodulation synchronization observed across separations of 1cm and 1.4cm is not solely due to volume conduction, and that the search for coherent synchronization in human gamma band ECoG needs to be at a smaller spatial scale.


Spine space synchronization (SSS) of neuronal ensemble activity of the human brain was investigated. Golgi electrodes were implanted in the deep structures for therapeutic purpose. The impulse activity of 14 distant ensembles were recorded simultaneously. It was established that there were some SSS for definite phases of psychological tests. This investigation was completed by chronic experiments. Monkeys (M.multiluata) were trained to differentiate colored geometric figures. The impulse activity of 6-10 neuronal ensembles was recorded simultaneously in associative, temporal and frontal cortex of the animals during their operant conditioning. It was show the existence, the reliability and the importance of SSS. There was a non linear correlation between discharge frequency changes of impulses and spike’s synchronization. The experiment showed that SSS correlated with definite phases of the monkeys’ behavior. The interaction of neuronal processes which have been investigated allow that such space interaction as SSS could be a special mechanism for quick short phases of integral mental processes in the primate brain and that spatial – temporal information stimulations by means of many implanted electrodes lead to modify behavior.


The goal of this experiment was to replicate and extend previous work suggesting that operantly conditioned changes in the amplitude of the prestimulus evoked potential (SEP) alters pain sensitivity. The SEP, R3 reflex, compound nerve action potential (CNAP) and magnitude rates were calculated by stimulation of the median nerve. Twenty-one subjects (conditioning group) were rewarded for increasing (up-training) and decreasing (down-training) the P200 peak of the human somatosensory evoked potential (SEP) alters pain sensitivity. The SEP, R3 reflex, compound nerve action potential (CNAP) and magnitude rates were calculated by stimulation of the median nerve. Twenty-one subjects (conditioning group) were rewarded for increasing (up-training) and decreasing (down-training) the P200 peak of the human somatosensory evoked potential (SEP) alters pain sensitivity. The SEP, R3 reflex, compound nerve action potential (CNAP) and magnitude rates were calculated by stimulation of the median nerve. Twenty-one subjects (conditioning group) were rewarded for increasing (up-training) and decreasing (down-training) the P200 peak of the human somatosensory evoked potential (SEP) alters pain sensitivity.玛丽亚

The SEP, R3 reflex, compound nerve action potential (CNAP) and magnitude rates were calculated by stimulation of the median nerve.
412.3
THE WISCONSIN CARD SORTING TASK SHOWS LITTLE SENSITIVITY OR SPECIFICITY FOR THE FRONTAL LOBE. Z. Carmanast*, M. P. Costa, and C. Damasio, Department of Radiology, University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

A new test, subjects must choose one card at a time from one of four decks. In two decks, choosing a card is followed by a high gain of play money, but at unpredictable times, the second deck is completely empty. In the other two decks, the immediate gain is smaller but the future loss is also smaller. After sampling and encountering losses in each deck, normal subjects begin to avoid the decks with high immediate gain (bad decks). Patients with right prefrontal-lobe lesions also show an avoidance strategy, but never reach the same level of strategy as do normal subjects. In the bad decks, there is no correlation between the amount of money won or lost and the frequency of a card being selected, which suggests an inability to use the results of the previous trials to predict future outcomes. In addition, the patients show a greater tendency to avoid the bad decks, which may be due to a reduced ability to predict future outcomes.

412.4
NON-CONSCIOUS AUTONOMIC SIGNALLING ADDRESSES THE AVOIDANCE OF RESPONSES WITH NEGATIVE AFFECTIVE CONSEQUENCES. D. Damasio, A. R. Damasio, and D. Tranel, Dept. of Neurology, Univ. of Iowa, Iowa City, IA, 52242.

After sampling and encountering losses in each deck, normal subjects begin to avoid the decks with high immediate gain (bad decks). Patients with right prefrontal-lobe lesions also show an avoidance strategy, but never reach the same level of strategy as do normal subjects. In the bad decks, there is no correlation between the amount of money won or lost and the frequency of a card being selected, which suggests an inability to use the results of the previous trials to predict future outcomes. In addition, the patients show a greater tendency to avoid the bad decks, which may be due to a reduced ability to predict future outcomes.

412.5
FRONTAL LESIONS AFFECT PERSISTENT AND MIRROR DRAWING PERFORMANCES. ML. Chouinard*, L. Bouleau, Lab. de Neurosciences de la Cognition, Université du Québec, Montréal, QC, HEC 3P8.

As part of a larger study of the role of the frontal lobes in sensori-motor performances and learning, we examined performance of patients with frontal lesions in two tasks requiring continuous regulation of movement on the basis of sensory information. We tested 5 patients with a unilateral frontal lobe lesion (FL) and 8 patients with a unilateral temporal lobe lesion (TL) on two sensori-motor tasks: 1) Rotary Pursuit and 2) Mirror Drawing. In the Pursuit task, subjects were asked to keep a light dot moving at a constant speed (15rpm, 30rpm, or 45 rpm) around a circle in three 20 sec. trials. In the Mirror Drawing task, the subjects had to trace a starlike path seen through a mirror. On the Rotary Pursuit task, FL spent less time on the target than TL at 30 and 45 rpm, suggesting a deficit in movement correction. On the Mirror Drawing task, FL showed longer total tracing time although their performance without the mirror was equivalent to that seen in TL. This slowing was mainly due to more frequent episodes of non-progressive tracing (jitter) for FL than for TL. However, line-crossing errors were not more frequent in FL. The performance of FL on both tasks suggests a difficulty in the control of movement under sensory guidance, especially under challenging conditions.

412.7
LOSS OF GLOBAL PROCESSING AFTER RIGHT HEMISPHERE ATROPHY. Alessandra Schievetto,1 Johannes Stauder,2 Laurent Mottron,2 Philippe Robecky3 and Marcie Lassonde1
1 Department of Psychology, Université de Montréal, and 2 Ste-Justine Hospital for Children, Montréal, Quebec, Canada.

The purpose of this study was to investigate the global and local processing abilities in a patient with a right hemispheric atrophy restricted to the temporal lobe. This atrophy resulted from a viral infection, herpes encephalitis, when the patient was 9 years old. As a result of this illness, the patient became prosopagnosic, color agnosic and presented evidence of associative visual agnosia. Recent MRI (Jan. 1993) revealed discreet left temporal lesions. The patient suffered from a global temporal atrophy, including the hippocampal region. In the present study, the patient was tested on two reaction time tasks in which hierarchical processing was manipulated: a global-local detection task and a figure-parsing task. Ten age-matched controls were also tested on the same tasks. Analysis of A.R.'s response accuracy shows that she could never distinguish between items. Moreover, reaction times in the global-local task show that, unlike normals, she did not have interference effect from the global to the local level. Indeed, her reaction times were slowed when congruent stimuli were presented (local vs. global level). Similarly, in the parsing task, she did not show the usual advantage for Gestalt fit items. In contrast to normals, her reaction times were significantly slower for the Gestalt items. These data suggest the involvement of the right temporal lobe in global processing. Furthermore, they may offer insight into the deficits resulting from visual agnosia.

412.8
FRONTAL LESIONS INCREASE ATTENTIONAL SUPPRESSION IN RAPID SERIAL IDENTIFICATION. P. Richter, M. Legue, Lab. de Neurosciences, Université du Québec, Montréal, QC, HEC 3P8.

While attentional problems have long been associated with frontal lesions in humans, the mechanisms underlying these deficits are still poorly understood. We have previously shown that frontal patients can have problems finding target stimuli in sequences (Richter et al., 1993) but it is not clear whether the processes of target detection or identification are different in frontal patients. To test this, we examined the effect of a first identification on a subsequent one in six patients with frontal lesions and six normals. Subjects were asked to name the two white letters embedded in rapid sequences of 15-24 black letters presented centrally on a video monitor at rates of 6 or 8/sec. After 20 practice trials, 60 sequences were presented in which the first target letter (T1) appeared randomly at times 9 through 15 and in which the second target letter (T2) appeared randomly at positions 1, 3, 5, or 7 following T1. Both frontals and normals showed near perfect identification of T1. In the case of T2, normals showed near perfect performance at all positions while frontals showed a poorer identification at positions T2 at T1+1 and T1+3 (50% correct), but not at T1+5 and T1+7 at a rate of 8 letters/sec. Frontals did not show this deficit at the slower presentation rate. These results indicate that the deficit in frontal patients may be due to pronounced or longer lasting interference on subsequent processing in frontals than normals.
413.9


We previously reported that localization of sound sources in hemispherectomized patients was less accurate than that of the controls in the homolateral hemisphere, and we hypothesized that this was due to a lesion in the midbrain. Moreover, their performances obtained with fixed sources were generally more precise than those obtained with moving sources (Neuropsychologia, 1994 in press). In order to precisely evaluate the effects of the hemispherectomy on free-field sound localization performances, the present study examined response accuracy to auditory targets in one patient, shortly before and 6 months after a functional right hemisecotomy was performed. We also evaluated the performances of three matched controls. Listeners reported sound positions by pointing with their dominant hand to the apparent sound location in an anechoic chamber. Two conditions were tested: (i) localization of a fixed-sound source and (ii) localization of the beginning and the end of a simulated moving stimulus. Prior to the operation, the patient was performing almost as well as the controls when fixed sources were used, but was significantly worse when moving sources were used. After the operation, the patient’s responses to fixed sources were less accurate than those of the controls in all positions tested in the hemifield contralateral to the removed hemisphere. Unexpectedly, the patient was now able to perform the moving task. These results are discussed in terms of the effects attributable to the surgery per se and they suggest the existence of a differential involvement of cortical and subcortical structures in the processing of stationary and moving sounds (supported by FCAR and CRNG).

413.11

VISUOSpatial ATTENTION SHIFT IN CEREBELLAR DISORDER. S. Yamaguchi1, H. Tsuchiya and S. Kobayashi, Third Div. of Internal Medicine, Shimane Med. Univ., Izumo, 693 Japan.

Cerebellar patients are known to be suggested to be involved in attentional shift mechanism. The present study investigated contributions of cerebellum on visuospatial attentional ability in a trial-by-trial cueing task involving covert orientation of visual attention. Event-related poten-
tials (ERPs) and reaction times (RTs) were measured in patients with cerebellar degenerative disorders and age-matched controls. The cerebellar group had slower RTs to both valid and invalid targets. The effects of cue validity on RTs were larger for the cerebellar group in both the central and peripheral cue experiments, suggesting efficiency of the attention shift process in the cerebellar group. ERP data over the cue-target interval (i.e. 800msec) in the central cue experiment demonstrated comparable effects of voluntary attention shift in both groups, the effects which were reflected in negative potential shifts at contralateral parietal and temporal sites in the early stage and central and frontal sites in the late stage. Peripheral cues also generated comparable effects of the early automatic and late voluntary attentional shift on ERPs in the cerebellar and control groups. The only difference in ERPs was CNV amplitudes, which were significantly diminished in the cerebellar group. These results suggest a preserved ability of visuospatial attention shift in cerebellar disorder.

413.12


Recent research in the field of intelligence has witnessed a resurgence in the search for brain correlates of intelligence. The current study examines two such explanations: brain volume and nerve conduction velocity. This study is composed of two phases. In Phase I, 40 healthy adult female subjects (ages 20 to 30 years) underwent magnetic resonance imaging (MRI) and nerve conduction velocity (NCV) procedures. It was found that brain volume was significantly correlated with IQ (r=.395, p<.05), but that NCV measures (taken along the median nerve and ulnar nerve) were not correlated (r=.12 and r=.02, both n.s.). A reanalysis of this and past NCV findings indicated a possible sex difference with NCV differing to be equal in males but not in females. Finally, it was found that brain volume and NCV were not correlated (r=.10 to .19, all n.s.). Phase II of this study, which is currently under way (36 subjects collected to date, with new subjects being obtained at the rate of about 3 per week), expands the study with a sample of healthy adult male siblings. Beyond a simple replication, Phase II uses MRI procedures that allow for advanced multi-exponential calculations of white matter relaxation times in addition to the brain volume, and therefore additionally in addition to peripheral, NCV. The use of siblings allows for assessment of within as well as between family effects, and so can illuminate the type of genetic and/or environmental factors at work in obtained correlations.

414.12

CENTRAL AUDITORY PROCESSING IN A FAMILY WITH 'DYSSARThIA'. D. M. Daly, Box 210685, Dallas, TX 75211-0685.

'Dyssarthria' includes various disorders involving control of movements in speaking. Using sets of computer synthesized sounds we examined auditory processing in a 6 yr. old boy referred with a diagnosis of 'functional dysarthria' and seven other members from three generations of his family. All had appropriate audiometric thresholds for age. One sibling, mother, and maternal grandmother reported similar problems: their speech is difficult for unaffected individuals to understand, yet they understand each other with less difficulty; they have difficulty drawing circles, turning a screwdriver/nothing hair curlers, or pedaling a bicycle; they are athletically challenged; they also have difficulty 'carrying a tune'. Two generations have received school based speech therapy; all three generations reported that speech improved spontaneously at 10-11 yr. All performed at or above grade level on core subjects. Unaffected individuals are free of these problems.

On sets of time varying synthesized sounds, neither proband, mother nor maternal grandmother distinguished B/D. Mother and maternal grandmother reported GV as vocable and yet with clear transition 20-30 msec less than controls. The affected sibling also differed significantly from controls. On sets of frequency varying sounds (BDG) mother and sons were less aberrant. Paternal grandmother, father, paternal grandfather and uncles were unaffected. There were no apparent transitions. Concurrent testing of affected and unaffected members confirmed marked perceptual differences; LRCS measures of divergence from individual and control composites substantiated these findings. Distribution in the pedigree is compatible with autosomal dominant expression.

412.15 A VERBAL ABSTRACTION DEFICIT IN MULTIPLE SCLEROSIS. W. W. Rebusck*, K. A. Harris, G. E. Bianco, R. H. Paul and E. L. Wilkins. Dept. Psychiatry & Behavioral Sciences, University of Oklahoma Health Sciences Center, P.O. Box 28901, Oklahoma City, OK 73190.

Impairments on nonverbal tests of abstraction by patients with multiple sclerosis (MS) have been reported frequently, but findings on verbal abstraction tasks are mixed. To examine the status of verbal reasoning in comparison to nonverbal reasoning in MS we administered the Shipley Institute of Living Scale (SILS), the Visual and Card Sorting Test (VCST), and a shortened version of the Free Sorting part of the California Card Sorting Test (CCST) to 100 MS patients and 32 age- and education-equated control subjects. The MS group achieved lower scores on the VCST, lower correct sorts on the CCST, and lower scores on the Abstraction scale from the SILS than did control subjects. Patients also scored lower on the Vocabulary Scale from the SILS, but because they also attained lower Conceptual Quotients, their poorer performance on the verbal abstraction cannot be attributed solely to lower verbal ability. Persuasive responding by patients was clearly elevated on the VCST, marginally increased on the CCST, and did not occur on the SILS, suggesting that difficulties in generating and identifying concepts are the major reasons MS patients often have difficulty on tests of problem solving or abstract reasoning.

Supported by a grant from the Oklahoma Center for the Advancement of Science and Technology.

412.19 DOES IMPAIRMENT OF WORKING MEMORY PRECEDE DEVELOPMENT OF DEMENTIA IN EARLY ALZHEIMER'S DISEASE? S. Martha, H. Chenow*, H. Bergman, N. Phu, Bloomfield Center for Studies in Aging, Lady Davis Institute, Jewish General Hospital, Montreal, Quebec, Canada.

Working memory (the store capable of holding information in memory while another cognitive task is performed) is impaired in subjects with dementia of the Alzheimer type (DAT) compared with elderly control subjects. We wished to examine whether a deficit in memory impairment may be demonstrable in elderly subjects not yet meeting criteria for dementia but having Age Associated Cognitive Decline (AACD).

Working memory was assessed with the Brown/Peterson task on 15 DAT, 17 AACD, and 12 elderly control subjects. On this task, consonant trigrams (three letters presented at once) were recalled over intervals of 3s, 5s, 10s and 20s. During the interval the subject is either allowed to rehearse the material with no distraction or is disrupted with subsidiary tasks (articulatory or digit rehearsal).

The results indicated that the average percentage of trigrams recalled by the three groups differed significantly (F = 9.20, P = .001). DAT subjects showed a moderate decline after 5s (35%) and 10s (42%) delay intervals, and the AACD subjects showed a mild decline at both the 5s (83%) and 10s (77%) interval. Elderly subjects showed a very slight decline at both conditions.

The percentage of trigrams recalled with digit reversal distraction after 5s indicated that the control subjects never showed greater than a 20% decline from baseline performance. 4/17 of the AACD subjects, and 10/15 of the DAT subjects demonstrated a decline below this performance level. These results demonstrate that working memory is not only impaired in DAT, but also in AACD subjects.

It is known that up to 2/3 of AACD patients eventually go on to dementia. Since working memory impairment can be demonstrated in a percentage of the AACD subjects it is possible that this might be a cognitive marker for future development of dementia.

412.20 IMPAIRED AUTONOMIC RESPONSES TO EMOTIONALLY SIGNIFICANT STIMULI IN ALZHEIMER'S DISEASE. C. C. Chu*, D. Tran, A. R. Damasio. Dept. of Neurology, Univ. Iowa, Coll. Med., Iowa City, IA 52242

Pathological changes in Alzheimer's disease (AD) selectively involve layer V neurons in Brodmann's area 25, posterior orbitofrontal cortex, and anterior insula, which project directly to subcortical autonomic centers. Disruption of these cortico-autonomic projections would be expected to compromise autonomic responses. We hypothesized that autonomic responses elicited by emotionally significant pictures would be impaired in AD patients. Color slides with high (target) or low (control) emotional significance were shown for 6-8 s while each stimulus was presented. Three to five target and control stimuli were presented in randomized order. The P300 components to the target and novel stimuli. Topographical maps of activity to the novel stimuli show a pattern of frontal activation in children not seen in adults. Results are compared to previous work with infant subjects and are discussed in terms of differences in attentional capacity, as well as the role of hippocampal and frontal lobe development in mediating the novelty response.

412.16 DISORDERS OF PROSODY IN PARKINSON'S DISEASE. H. Cohen*, Laboratory of Cognitive Neuroscience, Université du Québec, P8 8888, Station A, Montreal, QC, Canada H3C 3P8

Disturbances of intonational patterns of speech are some of the most common signs in Parkinson's disease (PD). If deficits in movement planning are responsible for this deterioration, we should then observe greater impairment in PD as the speech production tasks become more difficult. In this study we attempted to determine whether PD patients are more impaired, relative to controls, in the production of simple vowel syllables in increasingly more complex intonation contexts. Twenty non-demented PD patients (mean age = 68.6) and 15 controls matched for age and education (all subjects ≤ 75 yr) and no other neurologic or psychiatric disorder were asked to produce the vowels (a, e, i, o, u) in four intonation conditions: stable contexts (low and high intonation) and two unidirectional change contexts (ascending and descending intonation) for a period of five seconds. Subjects were recorded individually in a soundproofed room and speech was then digitized to extract indices of fundamental frequency (F0). ANOVAS were conducted on F0 measures, for each intonation condition, to determine the extent of group differences. Except for the low stable intonation condition, analyses revealed main effects of Group, suggesting a significantly more restricted F0 range in PD patients when producing vowels in stable high, as well as in ascending and descending intonation conditions (p < .001). The results suggest that the subcortical structures affected in idiopathic movement disorders are also involved in disorders of prosody. These structures are implicated in deficits in planning and sequential execution of movement and cognitive acts.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
413.3 FACILITATION OF AMPA RECEPTOR ACTIVATION INCREASES CLASSICAL FEAR CONDITIONING IN RATS. M. Rogan*, U. Stubli, J.E. LeDoux. Center for Neural Science, New York University, NY, NY 10003.

Rogan and LeDoux have previously shown that AMPA receptor agonists facilitate fear conditioning.


The authors examined the effects of ETOH pretreatment on classical conditioning and found that it selectively impairs the acquisition of contextual conditioning.


The study investigated the role of perirhinal cortex lesions in disrupting classical fear conditioning.

413.6 MEDIAL ORBITAL CORTEX LESIONS INCREASE RESISTANCE TO EXTINCTION BUT DO NOT AFFECT ACQUISITION OF FEAR CONDITIONING. M.A. Morgan* and J.E. LeDoux. Center for Neural Science, New York University, NY, NY 10003.

The authors found that lesions of the medial orbital cortex increase resistance to extinction but do not affect acquisition of fear conditioning.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
SEPTAL LESIONS POTENTIATE FREEZING BEHAVIOR TO CONTEXTUAL BUT NOT TO PHASIC CONDITIONED STIMULI.

P.D. Sparks* and J.C. LeDoux. Department of Psychology and Center for Neural Science, New York University, NY 10003.

Damage to the hippocampal formation interferes with the conditioning of fear responses to contextual but not phasic conditioned stimuli. Given the anatomical similarities and functional relationship between the hippocampus and the septum, we examined the effects of septal lesions on the conditioning of fear reactions to phasic and contextual stimuli. In the first experiment, rats with electrolytic lesions (n=9) or sham operations (n=6) were examined. Thirty days after surgery, blocks of two conditioning trials consisting of a tone (10kHz, 75 dB, 20 sec) paired with a foot shock (500 msec, 0.5 mA) were presented on two consecutive days. Tone alone trials were presented each day thereafter until extinction criterion was met. The amount of freezing elicited by the context but not by the tone was greater in the septal lesioned animals than in controls. The lesions involved the lateral and dorsoventral hippocampus.

Evidence from these experiments suggests that the hippocampal septal connections are necessary for the expression of contextual fear conditioning.

Supported by NSF2099464, MH37974, and MH09596.

HIPPOCAMPAL LESIONS BLOCK LATENT INHIBITION BUT NOT NEGATIVE TRANSFER BY A NON-NMDA RECEPTOR MEDIATED PROCESS. S. J. Young*, S. Maren and M. S. Fanselow, Dept. of Psychol., Univ. of Cal., Los Angeles, CA 90024-1563.

Latent inhibition describes a Pavlovian learning deficit caused by preexposure to the conditional stimulus (CS). Negative transfer is also a learning deficit but is caused by prior pairing of the CS with a weak unconditional stimulus (US). The two phenomena have traditionally been considered the result of a similar CS processing deficit. Evidence that both negative transfer and latent inhibition are context specific support this speculation. Latent inhibition is reversed by hippocampal lesions but the hippocampus does not block negative transfer. We have examined Experiment 1 examines the possibility that transfer is hippocampus dependent while latent inhibition is reversed by hippocampal lesions but the hippocampus does not block negative transfer. These results provide evidence that latent inhibition and negative transfer are different. The second set of experiments examined the role of NMDA receptors in latent inhibition. Application of a competitive NMDA receptor antagonist (APV, 5 µg/μl) had no impact on hippocampal and context dependent latent inhibition. These results are surprising because NMDA receptor mediated processes in the hippocampus are important for learning to recognize contextual cues. Supported by NIH 1F31MH10380-01 (SLV) and NSF BNS 9008820 (MSF).

SEX DIFFERENCES IN HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) AND PAVLOVIAN FEAR CONDITIONING IN RATS. M. S. Fanselow*, S. Maren and B. DeOca, Department of Psychology, University of California, Los Angeles, CA 90024-1563.

An emerging body of evidence indicates that the hippocampus is required for some forms of classical conditioning, particularly Pavlovian fear conditioning to contextual stimuli (CSs). Lesions placed in the fornix, dorsal hippocampus, or entorhinal cortex produce marked impairments in contextual fear conditioning. Recently, there has been a focus on what aspects of experience are critical for the acquisition and expression of contextual fear conditioning. In the current study, we examined the effects of hippocampal lesions on the acquisition and expression of contextual fear conditioning. We found that hippocampal lesions impaired the acquisition of contextual fear conditioning but had no effect on its expression. These findings support the idea that the hippocampus is necessary for the acquisition of contextual fear conditioning but not for its expression.

Supported by NSF grant BNS-9008820 to MSF.

C-FOS mRNA EXPRESSION FOLLOWING FOOTSTOCK STRESS AND CONTEXTUAL FEAR CONDITIONING. J. B. Rosen 1, M. S. Fanselow 2, S. L. Young 3 and S. Maren 1. 1Biological Psychiatry Br., NIMH, Bethesda, MD 20892, 2Psychol. Dept., Univ. of Cal, Los Angeles, CA 90024-1563.

Rats display conditional freezing behavior to contextual cues associated with footstock. Although the amygdala plays a prominent role in the expression of fear, little is known about its function in fear conditioning. We examined the role of the amygdala in fear conditioning by determining the effect of amygdaloid lesions on the expression of contextual fear conditioning. We found that lesions of the amygdala produced a complete and selective block of contextual fear conditioning.

Supported by NIMH 1F31MH10380-01 and NIMH grant MH-39786 to MSF.

DIFFERENTIAL EFFECTS OF VENTRAL AND DORSAL PERIAQUEDUCTAL GRAY (PAG) LESIONS ON DEFENSIVE RESPONSES OF RATS TO CATS, SHOCK AND TASTE AVERTION. B. DeOca, J. P. DeCola, J. L. Treadway and M. S. Fanselow. Department of Psychology, University of California, Los Angeles, CA 90024-1563.

Anatomical structures within the midbrain have been implicated in fear-related and defensive behavior. The present experiments examined the effects of lesions of the midbrain that included damage to the ventral portions of the periaqueductal gray (PAG) on defensive freezing responses to rats of the presence of a cat. Lesions that spared the ventral PAG did not affect freezing to a cat even if they produced extensive damage to the dorsal PAG. These results support the idea that the PAG plays a role in defensive response to rats of the presence of a cat. Lesions that spared the ventral PAG did not affect freezing to a cat even if they produced extensive damage to the dorsal PAG. These results support the idea that the PAG plays a role in defensive response to rats of the presence of a cat.
LESIONS OF THE AMYGDALA BLOCK CONDITIONED EXCITATION BUT NOT CONDITIONED INHIBITION OF EAR NOISE-INDUCED PAIN-POTENTIATED STARTLE.

W.A. Falle,* A.M. Davis, Dept. of Psychiatry, Yale Univ. Med. Sch., 34 Park St., New Haven, CT 06508.

Although much is known about the neural systems responsible for the acquisition and expression of conditioned fear, little is known about the neural systems responsible for the inhibition of fear. We have recently described a behavioral procedure for producing conditioned inhibition of fear-potentiated startle effect. Following training in which a light is repeatedly paired with shock (i.e., shocks) and a light-noise compound stimulus (i.e., light + shock), the animals acquire the ability to inhibit fear-potentiated startle to the light in a noise–light summation test (Falle and Davis, 1993; Neuroscience. Aflr. vol. 19, p.172, 115.6).

Amygdala lesions have been shown to disrupt both phase and magnitude of the fear-potentiated startle (Falle and Davis, 1993). When rats were given 15 light–shock pairings on each of 2 days, followed by a light-alone test on the third day, the magnitude of the startle response was increased. The lesioned rats showed a larger increase in the startle response magnitude than did the sham-operated controls. This is consistent with the hypothesis that the amygdala is involved in the production of conditioned inhibition of startle. The results are consistent with the hypothesis that the amygdala is involved in the production of conditioned inhibition of startle.

These results indicate that lesions of the amygdala critical for initial performance of fear-potentiated startle are not critical for the expression of conditioned inhibition.

DIFFERENTIAL EFFECTS OF AMYGDALA LESIONS ON ANTINOCICEPTIVE AND CARDIOVASCULAR RESPONSES TO CONDITIONAL AND UNCONDITIONAL AUDITORY STRESSORS. F.J. Hellstrom, G.L. & S.T. Tershner, Dept. of Psychology, University of Wisconsin, Milwaukee, WI 53201

Exposing rats to a tone that has been paired with shock or to a novel loud noise will produce a set of fear-related defensive behaviors which includes a simultaneous increase in arterial blood pressure (ABP) and decrease in sensitivity to pain. In the present study we directly compared the expression of conditioned and unconditioned fear responses using these two dependent measures within small groups of rats (9) which were presented to one of two groups: conditioned (C) or unconditioned (U). The conditioned group was exposed to a series of paired (or unpaired) presentations of a tone CS (60sec, 72dB) and shock and then retrained with a tone CS to the classical actions of a response. During subsequent testing all rats were exposed to both the CS and white noise (60 sec, 95dB) while recording ABP and radiometric heat flick (TF) latencies. Both auditory stimuli produced significant inhibition of TF in sham-operated animals that had received paired training. As reported previously, amygdala lesions blocked both fear of the CS and white noise while TBs also produced large time-dependent elevations in ABP with the noise-evoked response being considerably larger. However, animals with BLA damage that blocked hyperalgesia showed normal or potentiated ABP responses to both stimuli. These results suggest that neural systems responsible for the expression of learned versus unlearned fear responses may not differ although the control of ABP and TF may be dissociable within the amygdala.


Our laboratory has proposed a fictitious acoustic startle circuit that consists of the auditory nerve, pontine cochlear nucleus (VCN), an area ventral to the ventral cochlear nucleus (VCN, also known as the cochlear nuclei) of neurons in the spinal cord (Davis et al., 1992). The dorsal nucleus of the ventral cochlear nucleus (VCN, also known as the cochlear nuclei) consists of neurons in the spinal cord that are involved in the acoustic startle circuit more precisely. For example, lesions of VLN in the original study included the cochlear nucleus was primarily the auditory relay nucleus for the central auditory system and the central auditory system, including the central auditory system and the cochlear nucleus. Therefore, the present study re-evaluated the role of the structures previously implicated in the primary acoustic startle circuit using lesions bilateral, cochlear and central auditory systems, and the central auditory system.

Small lesions of VLN in the present study were used to eliminate the startle reflex, whereas large lesions included the entire area ventral to VLN and an area lateral to the ventral cochlear nucleus. Lesions were found in the ventral cochlear nucleus and in the area lateral to the ventral cochlear nucleus. In rodents, the lesioned neurons were found in the cochlear nucleus, named cochlear root neurons (CRN), that receive input from the cochlea, and have very little cytoplasm that consists of collagenous fibers terminating contralaterally in the ventromedial PRC (Lopez et al., 1993). Therefore, it must have been suggested that RN may be involved in an auditory paradigm (Verstraete & Fransen, 1990) in the acoustic startle reflex. Kainate lesions of CRN reduced the acoustic startle reflex by 90%, which is significant to the fact that these animals still retained the startle response and intact excitatory potentials recorded from the VCN using an 80-97 Hz white noise pulse or 10 Hz tone bursts.

Although the role of the VCN is yet to be fully determined, we now think that the acoustic startle reflex is more complex than it had been generally thought, consisting of only three synapses onto 1) RN, 2) neurons in the ventromedial PRC, and 3) neurons in the brain stem and spinal cord.


In a variety of mammalian species, the startle response elicited by a strong stimulus (the pulse) is reduced when preceded by a weak stimulus (the prepulse) which itself does not elicit startle. The phenomenon of "prepulse inhibition" (PPI) is often viewed as the mechanism of "memory gating" to the imperative stimulus (the pulse). For all groups the instrumental interval was varied between 15 and 30 sec (mean = 15 sec).

One hundred and five min. after the completion of the 9th trial session, all rats were subjected to another 10-trials session of active testing (no prepulse) with the trial interval varied between 30 and 60 sec (mean = 60 sec). As expected, the Prepulse group injected with MK-801 showed less prepulse inhibition than did the saline group. Additionally, the Prepulse group showed less freezing than did the saline group, consistent with the suggestion of NMDA receptors in the acquisition of conditioned fear. Responding to startle alone trials was similar in both the startle alone and the prepulse (excluding prepulse trials) paradigms. MK-801 appeared to increase responding to startle alone trials irrespective of paradigm. It is concluded that MK-801 produces deficits in prepulse inhibition but also in conditioned fear sensitization. Supported by ACS and NH&MRC.

MK801 ATTENUATES BOTH STARTLE PREPULSE INHIBITION AND CONDITIONAL FEAR SENSITIZATION. J. Crumley, T. Hargreaves, G. Pausini, and M. Neumaier, School of Psychology, The University of New South Wales, Sydney, 2052, Australia.

The non-competitive NMDA receptor antagonist MK-801 (MK-801) is known to decrease prepulse inhibition in the auditory startle paradigm (Munzchoth and Geyer, Neuropsychopharmacology, 1989, 2:299-308). The present study replicates and extends these observations by measuring freezing (an index of fear sensitization) in the prepulse inhibition paradigm as well as in a startle alone paradigm. Rats were administered with either MK-801 (0.1 mg/kg s.c.) or saline and presented 20 min later with 97 startleeliciting (122-dB) white noise stimuli. For rats in the prepulse inhibition paradigm, 48 of the 97 startle stimuli were preceded (100 msec earlier) by a low intensity (80-dB) white noise stimulus (the prepulse). For all groups the trial interval was varied between 10 and 20 sec (mean = 15 sec).

One hundred and five min. after the completion of the 9th trial session, all rats were subjected to another 10-trials session of active testing (no prepulse) with the trial interval varied between 30 and 60 sec (mean = 60 sec). As expected, the Prepulse group injected with MK-801 showed less prepulse inhibition than did the saline group. Additionally, the Prepulse group showed less freezing than did the saline group, consistent with the suggestion of NMDA receptors in the acquisition of conditioned fear. Responding to startle alone trials was similar in both the startle alone and the prepulse (excluding prepulse trials) paradigms. MK-801 appeared to increase responding to startle alone trials irrespective of paradigm. It is concluded that MK-801 produces deficits in prepulse inhibition but also in conditioned fear sensitization.

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413.10

EFFECTS OF ENTORHINAL CORTEX APOSIONS ON ODOR- AND CONTEXT-GUIDED FEAR CONDITIONING. T. Oros* and K.M. Schiller. Program in Biopsychology and Behavioral Neuroscience, Dept. of Psychology, Rutgers University, Newark, NJ 07102.

Previous work has demonstrated that hippocampal lesions abolish contextual fear conditioning but spare fear conditioning to a discrete tone CS (Phillips & LeDoux, J. Neurosci., 1992). The present study was conducted to determine the effects of damage to entorhinal cortex on contextual vs. odor fear conditioning using an odor as a CS. A group of male Sprague-Dawley rats received bilateral aspiration of the entorhinal cortex (n=12); a second group served as operated controls (SHAM, n=11). Ten days after surgery all subjects received 6 pairings of an odor (cis-3-Hexanol) and footshock (0.6mA, 2sec) in a distinctive operant chamber. On each of 6 trials the odor was presented for 30sec (delivered by means of a flow-dilution olfactometer and blown through the roof of the chamber) and the footshock overlapped with the final 2sec of odor delivery; the ISI was 4min. Twenty-four hours later each subject was placed in a novel operant chamber and measuring freezing behavior for 5min using a time-sampling method (5sec inter-sample interval). Conditioning to the odor was assessed by placing subjects into a context that was assessed by placing subjects into the operant chamber in which training took place and measuring freezing behavior for 5min using a time-sampling method (5sec inter-sample interval). Conditioning to the odor was assessed by placing subjects into a novel operant chamber and measuring freezing for 5min; the odor CS was presented during the second minute and remained in the chamber throughout the final 5min of sampling.

Histological verification of lesion placement has not yet been completed. However, preliminary analyses suggest that, using this paradigm, entorhinal cortex aspiration attenuates conditioning to both odor and the odor CS as indicated by significantly less freezing behavior for ENT subjects. This pattern of findings is consistent with the effects of hippocampal damage on contextual fear conditioning, but suggests that there may be a degree of sensory specificity in the role of the entorhinal cortex/hippocampal system in odor fear conditioning.

414.1


While the cerebellum is necessary and sufficient for the learning of the classical conditioning of the nictitating membrane in a delay paradigm, the hippocampus is required for the learning in a trace paradigm when at least 300 ms separate the onset of the CS from the US onset. However, the importance of the cerebellum in a trace paradigm is not well understood. While the interpositus nucleus is absolutely necessary for the performance of a previously learned trace conditioning task, its importance during the acquisition phase is not well understood. In an attempt to answer this question, we produced reversible inactivation of the cerebellum by muscimol infusion in the interpositus nucleus during the acquisition phase for a trace paradigm.

Male New Zealand white rabbits were implanted with a cannula in the interpositus ipsilateral from the stimulated eye. Rats were then trained on a trace paradigm (500 ms trace interval) and infused with muscimol before each training session. The rabbits were trained with muscimol infusion for 12 sessions after which they were infused with saline for the following sessions. The animals did not show any CRs during the first 12 training sessions and gradually developed CRs thereafter as would naive animals.

This work was supported by NSF: IBN-9110777 to MB and NSF BNS-8718300, NIH (NIA) AG05142 and a Sanko grant to RFT.

414.3


Numerous studies have demonstrated that during classical conditioning of the eyelid reflex, subjects learn to time the conditioned response (CR) so that the peak of the CR occurs at about the time the US is normally delivered. The present study was designed to investigate whether the red nucleus might play a role in accurate timing of the CR. Two groups of rabbits were implanted with cannulae aimed at the contralateral red nucleus (RN). Following recovery, all rabbits received 6 training sessions in which a tone CS (650 ms) was paired with a concomitantly airpuff US (100 ms) so that the interstimulus interval was 550 ms. All rabbits were then given 7 more training sessions in which the ISI was shifted to 200 ms. Prior to the first 3 of these 200 ms ISI sessions, one group was infused with muscimol into the RN while the other group received vehicle infusions into the RN. Consistent with previous studies, all rabbits learned to time the CR during the first 6 sessions so that the peak occurred at about 500 ms after US onset (as measured on CS alone test trials). When switched to the 200 ms ISI, the rabbits which were infused with saline (n=8) readily learned to time the CR so that after the first session, there was a significant reduction in peak CR latency and by the end of the third session, the mean peak latency was 250ms. In marked contrast, the rabbits infused with muscimol into the RN (n=6) performed no CRs during the 3 induction sessions with the 200 ms ISI (and none of the 3 induction sessions without muscimol) the mean peak CR latency did not differ from the peak latency obtained during the last session with a 550 ms ISI (session 6). During subsequent sessions, these rabbits learned to time the CR at the same rate as the saline infused controls. These data indicate that inactivation of the RN (which previous studies have shown to have no effect on the ability to acquire the CR with a 250 ms ISI) disrupts the animal's ability to properly time a previously learned CR. [Support: NSF (BNS-9213069), NIH (AG05142), Sanka, NIA].

414.2


Classical conditioning of discrete motor responses of the facial and neck musculature has been shown in rabbits implanted with stimulating electrodes in cerebellar cortex. Following threshold measurements for the elicitation of discrete motor responses, subthreshold stimulation was used as the CS and suprathreshold stimulation through the same electrode served as the US. In this paper we report on a discrimination conditioning following paired (but not randomly unpaired) CS-US trials, extinction following CS-alone trials, and reacquisition with savings, as well as a significant postretraining increase in the lateral cortical excitability. The results are consistent with the hypothesis that the cerebellar cortex plays an important role in the regulation of motor behavior. These findings indicate that the cerebellar cortex is involved in the regulation of motor behavior, in that the cerebellar cortex is involved in the regulation of motor behavior. These findings indicate that the cerebellar cortex is involved in the regulation of motor behavior, in that the cerebellar cortex is involved in the regulation of motor behavior. These findings indicate that the cerebellar cortex is involved in the regulation of motor behavior, in that the cerebellar cortex is involved in the regulation of motor behavior. These findings indicate that the cerebellar cortex is involved in the regulation of motor behavior, in that the cerebellar cortex is involved in the regulation of motor behavior.
414.6 MULTIPLE UNIT RECORDINGS FROM THE TRIGEMINAL COMPLEX.
EYEBLINK CONDITIONED UNIT RESPONSES DURING INTERPOSITOR OR RED NUCLEUS INACTIVATION VIA COOLING. Robert E. Clark, Elizabeth B. Gehl, and David G. Lavond. Departments of Psychology and Biological Sciences, University of Southern California, Los Angeles, CA 90089-2520.

The trigeminal complex is of considerable interest to researchers of the conditioned eyelink response for primarily two reasons. (i) The conditioned response pathway involves the trigeminal system. (ii) Other research has suggested that the trigeminal complex may also participate in the generation of the behavioral conditioned response or even in the formation of the essential plasticity for the learned response.

Recordings were obtained from the trigeminal complex with the aid of a moveable electrode in rabbits classically conditioned with a tone-conditioned stimulus (CS) and an airpuff unconditioned stimulus (US). Units were examined over a block of 9 trials during normal training. These unit recordings were then examined over a block of 9 trials during reversible cooling lesions of either the interpositus or red nucleus.

Unit responses to the CS and US were frequently observed as were responses that modeled the learned behavioral response. The model responses were found in various regions of the trigeminal complex, but most prominently in the principal sensory nucleus and pars oralis of the spinal trigeminal. Cooling either the interpositus or red nucleus completely abolished the unit models but did not abolish the responses evoked by the CS or US. We conclude that the learning related activity in the trigeminal complex is driven by the interpositus via the red nucleus.

(Supported by NSF BNS 8718500, ONR N00014-88-K-0112, and the McKnight Foundation, to R.F. Thompson.)

414.7 FUNCTIONAL LOCALIZATION AND NEUROANATOMICAL CONNECTIVITY OF NEURAL SUBSTRATES WITHIN THE ANTERIOR INTERPOSED NUCLEUS INVOLVED IN EXPRESSION OF THE NICTITATING MEMBRANE REFLEX.
S.A. Bartholomew, M.L. Webster, J.R. Bloom, V. Bracha-Barrow Neurological Institute, Phoenix, AZ 85013.

The purpose of the present study was to analyze the neuroanatomical and neurochemical connectivity of projections within the anterior interposed nucleus (AIN) which are critical for expression of the classically conditioned nictitating membrane reflex (NMR) in the rabbit. Animals were chronically implanted with a matrix of three guiding cannulae in the region of the AIN and then conditioned in the standard delay paradigm. A functional map of the AIN was reconstructed from the behavioral effects of systemic muscimol injection (20 mg in 50 nl) along the three injection tracks in trained animals. In each animal, the AIN subregion in which muscimol microinjections most effectively blocked the conditioned response performance was electrophoretically injected with antiserum (PHA-L) and retrograde (CTB) tracers.

The data indicate that besides the traditionally considered AIN connections with the red nucleus, the pontine nuclei, and the inferior olives, several other projections could also be relevant to AIN involvement in the NMR expression: 1) a direct afferent projection from the spinal trigeminal nucleus; 2) direct efferent connections to the mesencephalic periaqueductal and reticular accessory ocularis motor regions; and 3) an efferent projection to the pontine nuclei.

NIH Grants NS 30013 and NS 21958.


Stimulus-induced, perceptually related events are accessed in paired- and nocorres by recording the endogenously generated spatio-temporal patterns of the EEG. They take the form of amplitude modulation in spatial coordinates of a broadband spectrum, aperiodic carrier wave. This finding has been extended to characterize the evolution of such patterns across time and across sensory modalities. Arrays of 64 electrodes (8x8 at 0.79mm spacing) were fixed onto the epidural surface of either the prefrontal, visual, auditory, or somatosensory cortex of 12 rabbits classically conditioned to discriminate between pairs of stimuli (CS+ and CS-) not related to the cortical implant site. The 64-electrode tracings were decomposed by FFT, PCA, RMS, and spectral entropy analysis for 120ms/1/8second windows stepped at overlapping 20msSec intervals. Pattern differences were expressed as Euclidean distances in 64-space. Decomposition and successful classification of the EEG patterns indicated that sensory information is shared between all primary cortical sensory processing areas during lagged time periods after stimulus arrival. Long term EEG recordings have also shown that basaltic spatio-temporal patterns are inconstant and continually evolve at a constant rate corresponding to perceptual drift. A temporal frequency analysis of the EEG has demonstrated no relationship between periods where percept are formed and an increase or decrease in the power spectral density within narrow bands of the temporal spectra. We conclude that cortical function in perception is an endogenous process in which percepts are constructed, not filtered, by chaotic nonlinear dynamics. Funded by the National Institute of Mental Health - MH000686.
414.11 THE ABSENCE OF INTERHESMERIC COMMUNICATION IN THE NORMAL RABBIT, BY J. Suele Russell, Anatomy, Texas A&M University, College Station, TX, 77843-1114.

The presence of the role of the cerebral cortex to inhibit interhemispheric communication in normal rabbits. Normal rabbits (N=40) were used for visual interocular transfer (U.O.T.) using a variety of visual tasks in a two choice situation. They were first trained monocularly to criterion. Following this they were trained with the other eye. No signs of IOT were seen in any animal. All animals had to be rescued from chance and showed no savings. This suggests that the normal intact rabbit functions as a "split brain" animal, i.e. the left hemisphere does not communicate with the right hemisphere.

To test this, a group of rabbits were monocularly trained on a pattern discrimination task. After reaching criterion the animals were hemidescorticized such that half of them had the hemisphere contralateral to the trained eye and the others had the ipsilateral hemisphere lesioned. The results showed that following retraining the intact hemisphere was again established in the normal rabbit. Perfect retention of learning was found following ipsilateral hemidecortication; whereas no retention was evident when the lesion was contralateral.

These findings suggest that the contralateral optical projections play an important role in collateral inhibition of the ipsilateral projections and thus prevent the formation of a bilateral memory record. The lack of such a record would also explain the absence of U.O.T. in the rabbit. Hemidecortication prior to learning was found to disrupt this inhibitory mechanism. Such hemidecorticated rabbits when trained monocularly showed a bilateral memory record and perfect IOT. These findings argue strongly that the rabbit has a cortical inhibitory control over the extent interhemispheric communication required during monocular viewings.


In rats, eyelink conditioning develops dramatically between postnatal day 17 (PND17) and PND24 (Stanton, Freeman, & Stammer, 1992). Retaring with mitomycin-C (67-655) has been found to disrupt cerebellar development (Alvarez, 1969; Comp. Neurol., 120, 269-294). The current study was designed to investigate the effects of mitomycin-C on cerebellar maturation as a result of disrupted eyelink conditioning in adult animals (Thompson, 1988, JNVS, 11, 152-155). These observations suggest that the ontogeny of eyelink conditioning depends upon the maturation of the cerebellum. In the present study, we experimentally manipulated cerebellar maturation by exposing neonatal rat pups to the antimitotic agent methylazoxymethanol (MMAM) during cerebellar cortical neurogenesis. Pups were then trained with eyelink conditioning procedures at different ages. In Experiment 1, pups were given saline or MMAM (0 mg/kg) on PND17 and 25 days again. The results showed that this treatment impaired eyelink conditioning at all ages tested without affecting unconditioned responding or delayed alternation in a T-maze. This study suggests that cerebellar maturation is critical for the ontogeny of eyelink conditioning.


Previous work in our laboratory (Stanton, Freeman, & Shelley, 1992, Revue. Neurosci., 106, 657-665) has determined that the classically conditioned eyelink response in the rat appears during postnatal Day 17 (PND17) and PND24. The current study sought to explore the possibility that conditioning acquisition does occur at PND17, but cannot be expressed until a later age. Animals were trained in a Pavlovian eyelink preparation utilizing a CS and a neutral shock US. Trials of two different frequencies, 2.8 and 5 kHz, were used because some of our recent findings have indicated that CS frequency can be an important variable in the ontogeny of conditioned eyelink responses. A "savings" design was used in which acquisition on PND20 was assessed as a function of prior training on PND17. Group P-P received 300 trials of paired training on PND17 and again on PND20. Performance of this group was compared with that of control groups that received no treatment (N-P) or unpaired training (U-P) on PND17, followed by paired training on PND20. Two control groups (U-U) received no treatment on PND17 and unpaired stimulus presentations on PND20. Response acquisition on PND20 was found to differ reliably as a function of the animals' experience on PND17. Group P-P showed accelerated acquisition of the conditioned eyelink response on PND20 relative to group U-P and N-P, indicating that some unspecified associative learning occurred during paired training on PND17. A surprising finding was that unpaired exposure with the 2.8 kHz tone had a facilitatory effect on PND20 acquisition, while unpaired exposures with the 5 kHz CS resulted in a significant retardation of day 20 acquisition. These findings suggest that maturation of fibers afferent to the cerebellum may play a role in the developmental appearance of eyeblink conditioning in the rat.


The rat eyelink reflex is an attractive model system for studying associative learning. The neural circuitry underlying the last component (R1) involves a three-synapse arc between the 5th sensory nerve and the organizing cellucl (A.o.) muscle, which is responsible for the eyelid closure. In the rabbit, the nictitating membrane response can be associatively modulated by an amygdala-dependent mechanism (Weisz et al., 1992). We were interested in knowing more about the relationship between the amygdala and the eyelink reflex in the rat, which is more convenient for in vitro cellular neurophysiology.

We note that stimulation of the amygdala facilitates the R1 component of the EMG recorded in the c.o. muscle. Twelve rats were implanted with a bipolar nerve cuff placed around the 5th nerve to elicct eyelink and a monopolar stimulating electrode targeted at the amygdala. Both nerve cuff (SNCS) and electrical brain stimulation (EBS; 100-400 μA, 0.2-0.8 ms) of the amygdala resulted in a single biphasic square wave pulse. All electrode placements were histologically verified. We found that amygdal stimulation facilitation R1 maximally when EBS and NCS were presented simultaneously, suggesting that the projection from the amygdala to the site of reflex modulation must be fairly direct.

We are presently using confocal microscopy to trace projections from the amygdala to the facial nucleus, using in vivo injections of anterograde fluorescent tracers into the amygdala and tetramethylrhodamine into the nictitating memble muscle to retrogradely identify motorneurons in the facial nucleus (Lothius and Brown, 1993).

The avian hippocampus is similar to the rat hippocampus in that it processes various types of spatial information. Avian hippocampal neurons can also be used for spatial memory tasks in a variety of species, including pigeons, parakeets, and crows. In particular, pigeons (Columbia livia) have been used extensively to study spatial memory due to their large size, ease of handling, and relatively simple social structure. Pigeons are able to learn and remember a variety of spatial tasks, including finding food in a novel environment, navigating through a maze, and even distinguishing between different types of visual stimuli.


Previous research has indicated that some species of birds, such as the black-capped chickadee (Parus atricapillus), are able to orient themselves using the sun as a compass. This orientation is thought to be important for food-finding behaviour, as chickadees often need to locate food resources in their environment. To study this behaviour, the researchers fed chickadees with black sunflower seeds, which chickadees are known to prefer. The birds were then exposed to a circular arena where they were given the choice of flying towards a real sun or a simulated sun, which was positioned at an angle of 45 degrees from the real sun. The results showed that the chickadees were able to orient themselves using the sun as a compass, suggesting that they use the sun as a cue for orientation.


Several recent studies have described sex differences in the size of the hippocampus that are correlated with ecological differences in the use of space. Male pigeons and female brood-parallel pigeons possess different spatial representations compared to non-parallel pigeons and male pigeons, respectively. This difference in the size of the hippocampus is correlated with changes in the use of space and the ecological differences described correlations could be advantageous. Black-capped chickadees (Parus atricapillus) store food and retrieve their stashed caches using spatial memory for cache sites. The present study examined sex differences in caching, cache retrieval, and accuracy for cache sites. The results indicated that the sex differences in caching were due to the size of the hippocampus. This result suggests that the size of the hippocampus is correlated with the use of spatial memory and the ecological differences described in the use of space and sex.


In the forebrain of domestic birds several brain regions are involved in early filial imprinting. While the immediate hyperstriatum ventrale (MHV) and the hyperstriatum accessorium (LPO) are not involved in visual imprinting, a system of interconnected forebrain areas such as the hyperstriatum ventrale externum (HVE), the hyperstriatum accessorium (LPO) and the caudal neostriatum (Ndc) is involved in auditory imprinting. Since a natural imprinting object combines visual and acoustic features, we postulate that in the imprinting region there must exist, in which the two sensory modalities are processed on a higher associative level. By using 2-deoxyglucose (2-DG) autoradiography, we could localize the activation patterns of acoustically or visually stimulated chicks with a high contrast against the background of the visual control. We conducted two different imprinting procedures: i) individual chicks were imprinted on rhythmic 400 Hz tone pulses without presenting visual stimuli, ii) individual chicks were imprinted on a rotating red flashing light without presenting auditory stimuli. During the 2-DG experiment imprinted chicks of both groups were exposed to either the visual or acoustic imprinting stimulus, respectively. A region in the dorso-caudal part of the neostriatum, which we term dorsocaudal neostriatum (Ndc), was strongly labelled in the acoustically as well as in the visually imprinted chicks. In order to rule out that the activation of emotional stress during the 2-DG experiment, we investigated the activation patterns in naive, unimprinted chicks, which were exposed. Other brain areas in the region of the Ndc were activated, which was consistent with the idea that the Ndc is a key structure in the learning of imprinting.

(Supported by grant GSF 07 NB 06 of the BMFT)

414.21  Dopamine in Learning Relevant Forebrain Areas of the Domestic Chick: A Combined Immunohistochemical/Tracing Study M. Metzger, H.J. Böcher* and K. Braun. Federal Institute for Neurobiology, Braunschweig, 39118 Magdeburg & Dept. of Biology, University of Bielefeld, FRG.

As demonstrated by 2-deoxyglucose autoradiography, acoustically imprinted domestic chicks develop an increased metabolic activity of several telencephalic areas, including the medio-dorsal neostriatum/hyperstriatum ventrale (MNV), the lateral parolfactory (LPO) and the dorsal neostriatum caudale (Ndc). Since there is no evidence for dopaminergic input to these areas, it is likely that the dopaminergic activity is mediated by a different neurotransmitter. To study the effects of dopamine DA stimulation, we have used an antibody to DA (dopamine), which labels all dopaminergic neurons in the brain. We then incubated the brains with dopamine and examined the distribution of the antibody. The results indicated that dopamine stimulation had no effect on the distribution of the antibody, suggesting that dopamine DA stimulation has no significant effect on the distribution of the antibody.

(Supported by grant GSF 07 NB 06 & by the Deutsche Volkswagenstiftung)
415.1 ACCURACY OF SPATIAL NAVIGATION: THE ROLE OF PLATFORM AND TANK SIZE. C.F. Maciewicz* and R.M. Bauer. College of Pharmacy, Tobacco & Health, State University of New York College of Medicine, University of Kentucky, Lexington, KY 40504.

The Morris water maze task has great popularity for assessing both the cognitive processes and spatial ability of animals; it involves the most time-consuming task of spatial learning. However, little systematic attention has been given to the role of several major parameters (e.g., tank and/or platform size) which may provide very different "instructions" to the animal. Adult Sprague-Dawley rats were trained for 90 days of age under one of three conditions: a small (3cm²), medium (10cm²), or large (14cm²) escape platform in a standard 1.2m diameter tank. All animals (n=18) received 9, 8, and 4 training trials on three consecutive days followed immediately by a probe test trial on the third day. After a 10-hour interval, the animals received 4 additional training trials followed by a second probe test trial (the previous extraneous context). Platform size differed during acquisition—the most rapid decrease in latency was associated with the largest platform. All groups demonstrated a significant spatial bias during the test trial. In marked contrast to the training data, the lateral crossing measure, the zone on as index of spatial accuracy, was enhanced inversely with platform size. Additional experiments demonstrated the generality of this relationship between spatial accuracy and relative platform size (1:60 - 1:450) was invariant to tank size (Uffen, 1:20). All groups performed significantly worse than naive animals when tested in an equivalent platform. The results of these studies were interpreted as follows: since rats are able to overcome a sensory-motor deficit, they are able to learn spatial cues which are not available in the environment. However, the ability to learn spatial cues is influenced by the size of the platform, with larger platforms facilitating learning. This finding suggests that the Morris water maze task may be a useful tool for assessing spatial learning and memory in rats. Additional experiments are needed to determine the extent to which these findings are generalizable to other species of animals.

415.2 HIPPOCAMPAL LESIONS IMPAIR NEGATIVE PATTERNING, BUT NOT CONDITIONAL LEARNING, USING CONTEXTUAL CUES. D.M. Skinner* and D. van der Kooij. Dept. of Anatomy and Cell Biology, University of Toronto, Toronto, Ontario, M5S 1A8.

While performance on tasks employing spatial and contextual cues depends on the integrity of the hippocampus, we have previously shown that acquisition of conditional discriminations is not impaired by hippocampal lesions. Animals were trained on a conditional discrimination task that employed task aversiveness. Half of the animals were placed in a novel context (a distinct test box) prior to a flavor-LiCl pairing. On alternate days animals were exposed to another distinct context prior to the flavor-LiCl pairing. Aspirin lesions of the hippocampus acquired the task (learning to drink on safe context trials), but at a slightly slower rate than sham controls. We now report that the acquisition of hippocampal lesion of the hippocampus severely impair acquisition of a negative patterning task that employed the same cues. In a negative patterning task each of two cues is followed by an unconditioned stimulus when presented singly, but the unconditioned stimulus is withheld when both cues are presented together. We used a novel 0.1% saccharin solution and a distinct context as cues. Each of these cues alone signaled an injection of LiCl, but when presented together signaled the absence of LiCl. Both lesioned and sham animals acquired the conditional component of the task, consuming more saccharin in the rats novel context than in the home cage. However, the addition of context-LiCl trials impaired the performances of both groups. With further training, the lesion sham animals acquired the negative patterning task.


Contextual cues are important in determining performance in behavioral "interference" paradigms, such as extinction, in which the CS is associated with different outcomes in successive phases of the experiment. After extinction, contexts control performance by retrieving the current relation between the stimuli. This research examined the role of the hippocampus in mediating the effects of context and extinction in a shuttle box pairing in rats. Half the animals from each group were given CS alone trials in Context A (Cont A, Form A), half in Context B (Cont B, Form B). Testing was conducted in Context A. None of the groups differed with respect to pre-CS baseline responding, rates of conditioning, or extinction. Rats with fornix lesions (Form A) showed increased spontaneous recovery when compared to controls, but no attenuation of renewal. Suppression ratios (SEM) on the first test trial were: Control A = 044 (Cont B = 08 03), Form A = 41 (04), Form B = 16 04. In reinstatement, the animals responding to an extinguished CS recovered when the animal is given unsignaled presentations in the context of extinction. Animals were reassigned such that half received reinstating US presentations in the same context, half in a context different from extinction. Animals with fornix lesions (Form A) showed extinction in the extinction context did not show reinstated conditioned responding to the extinguished CS, whereas the comparable control group did (Cont A). Suppression ratios (SEM) on the first test trial were: Control Same = 16 06, Control Diff = 44 04 (Form Same = 37 03, Form Diff = 44 05). Results suggest that the hippocampal system plays a role in some, but not all, effects of context after extinction.

415.4 EVALUATION OF TIME OF DAY AS A CONDITIONAL CUE FOR RATS. A. Koerner*, R. J. Sutherland, G. M. Martin, B. J. McDonald, & S. Avery. Dept. of Psychology, University of New Mexico, Albuquerque, NM 87131.

We evaluate representational theories of conditioning which ascribe an important role to when and where an event occurs. We describe 4 discrimination experiments which show that rats' sensitivity to time of day should not be given a special role in theories of learning. Our data indicate that we should distinguish between two types of where: The place of training and the contextual cues present during training.

All experiments involve conditional discrimination training based upon place or time of day. The rats were trained that X, not Y, was correct at Time 1 or Place 1, and that Y, not X, is correct at Time 2 or Place 2. Experiment 1 shows that rats can use time of day as a conditional cue after extensive training if they are required to distinguish between a black and white cue in a M-shaped and that hippocampal damage abolishes the discrimination. Experiment 2 shows that rats do not learn to use time of day as a conditional cue when they are required to distinguish between a left and right turn. Comparison of a control group and the rats showed a win-stay/lose-shift strategy. Experiment 3 shows that rats alter a single reversal readily spatial location as a conditional cue when they are required to distinguish between a black and a white cue in a Y-maze and that hippocampal damage abolishes the discrimination. Experiment 4 shows that rats do not learn to use time of day as a conditional cue when they are required to discriminate between a left and right turn. Experiment 4 eliminates spatially differential response requirements using operant chambers. Experiment 4 shows that rats are capable of using time of day and contextual cues (presence or absence of a clear plastic floor) as conditional cues after extensive training which does not involve changes in location of responding, when they are required to discriminate between a tone and light in an operant chamber. These results question the notion that time is a general salient dimension in discrimination learning.

415.5 A VISUAL CUE ENHANCED BY SOUND IMPROVES SPATIAL LEARNING AND DECREASES RESPONSES TO DISTRACTION IN ANIMALS WITH HIPPOCAMPAL DAMAGE. E. Hedba-Rauer*, T. Briones, and B. Therrien.

The University of Michigan, Ann Arbor, MI 48109.

Spatial disorientation is caused by damage to the hippocampal formation (HPC). We previously reported that a single visual cue improves spatial learning of animals with hippocampal damage but does not improve cognitive performance by a simple environmental distractor. This study used a visual cue enhanced by sound to determine the effects of a multi-modal stimulus (cue) on cue navigation in the presence of a visual cue with an auditory cue. Both trends with HPC damage and females with unilateral damage were not impaired. However, the animals trained with bilateral lesions remained impaired on all measures (p<.05). After four days of testing with the cue, a distractor was introduced. In the presence of the enhanced cue, males with unilateral damage were not distracted performing as well as controls on all test days. In contrast, all females with HPC damage and males with bilateral lesions were more distractible than controls (p<.05). However, all animals were housed in the presence of the enhanced cue as compared to animals exposed to a single visual cue. We conclude that a visual cue enhanced by sound markedly improves spatial learning of disoriented animals. Importantly it eliminates or reduces the disruptive effect of a simple environmental distractor in animals with HPC damage.

415.6 RATS WITH HIPPOCAMPAL DAMAGE LEARN SPATIAL RELATIONSHIPS WHICH THEY CANNOT USE TO GUIDE NAVIGATION. R. J. Sutherland, G. M. Martin, C. Edwards, & K. Williams, Deps. of Psychol., Univ. of New Mexico, Albuquerque, NM 87131 and Memorial Univ. of Newfoundland, St. John's, NFLD, A1B 3X9.

Many theories of hippocampal function hold that its activity represents a mechanism of relational information. Medial temporal lobe amnesics demonstrate effects of prior learning about an episode if tested in indirect memory tasks, but not in direct tests of memory. We examined rats using two tests of spatial relational memory in the same environment; one direct (place navigation) and one indirect (disambiguation of exploration). Twelve rats were trained in the Morris water task to locate a fixed, hidden platform. Half of the rats received microinjections of a neurotoxin solution of kainate+colchicine. All rats were allowed to explore a dry pool in a new room in daily 5-min sessions for 37 days. Four object stimuli were present in the pool in fixed locations. Two probe trials were conducted: 1) the pool and 4 objects were rotated 180° relative to the room and 2) two of the objects were removed and the pool was tested for place navigation in the new and the old room. The rats with HPC damage were profoundly impaired in the place navigation in the new and old rooms. In contrast, control rats and rats with damage detected the changes in spatial relationships in probe trials, as shown by reliable increases in exploration. Thus, learning the layout of an environment survives HPC damage, although this information is not used for accurate navigation.
415.1
HIPPOCAMPAL DAMAGE IN RATS CAUSES RETROGRADE AMNESIA FOR PLACE NAVIGATION BUT NOT FOR OBJECT DISCRIMINATIONS. R.S. Astur*, D.G. Mump, M.P. Weisend, and R.J. Sutherland. Depts. of Psych. and Physiology, Univ. of New Mexico, Albuquerque, NM 87131.

In order to examine the effects of partial and complete hippocampal lesions on object and place memories, 29 rats were trained on a different object discrimination task each week prior to surgery. To examine place memory, each rat was trained in the Morris water task in two different rooms, 14 and 7 weeks prior to surgery. Bilateral hippocampal lesions were made using multiple intrahippocampal microinjections of ibotenic acid. Ten rats received complete hippocampal lesions, 10 rats received partial hippocampal lesions, and 9 rats received sham lesions.

Retrograde effects were assessed by testing all rats on the five object discriminations and the two pool problems that they had learned previously. Anterograde effects were assessed by teaching rats two new object discriminations and one new pool problem. For the previously learned object discriminations, there were no significant differences between the groups in retaining the discriminations nor in their accuracy in the first five trials of testing. For the new object discriminations, there were no significant differences between the groups in learning either new object discrimination. For the previously learned pool problems, both HFC groups were impaired relative to controls in finding the platform and percent of time spent in the correct quadrant during probe trials. Only the complete HFC group did not learn the new pool problem. These data indicate that HFC is important for navigating to places, but not for object discriminations.

415.9
OBJECT DISCRIMINATION IN NORMAL AND HIPPOCAMPUS-DAMAGED RATS. D.G. Mump*, D. Pretz, R.S. Astur, R.L. Klein, R.J. Sutherland, and G.M. Martin. Depts. of Psych, University of New Mexico, Albuquerque, NM and Memorial University of St. John's, NF.

We examined whether rats can recognize objects independently of viewing perspective. Normal rats and rats with bilateral ibotenic acid lesions of the hippocampus (HFC) were trained to discriminate between three object pairs. Initially rats were trained with the objects in one orientation revealed that the original discriminations were retained (Mean errors to criterion, 4.6 & 5.1 for HFC and normals rats, respectively). Changing the orientation of the objects during training disrupted performance (Mean errors to criterion, 16 & 14 for HFC and normal rats, respectively). The objects were then coated with acrylic to increase the similarity of the objects' surface properties, including odor. Rats that were trained with the objects in the original orientation revealed that the acrylic did not affect performance (Mean errors to criterion, 2.1 & 3.5 for HFC and normal rats, respectively). The final test revealed that switching the orientations of the acrylic-coated objects interfered dramatically with acquisition of the discrimination (None of the rats reached criterion; Mean errors, 78 & 65 errors for HFC and normal rats, respectively). Further tests were carried out to assess the use of spatial information when the correct solution of an object depended upon its spatial location.

Object discrimination performance in our task depends upon object orientation. Moreover, it may be that rats solve object discriminations by distinguishing between object pairs on the basis of a single visual or surface property.

415.11

Fimbria-fornix lesions cause hyperactivity and learning difficulty in rats. During the course of other studies investigating memory deficit in fornix-lesioned rats we had occasion to observe pronounced stereotypic behaviour. Specifically a cannulated knife was used to produce selective lesion to the fornix in 12 Long Evans hooded rats; 9 animals undergoing sham operation served as controls. As expected the animals with fornical lesions demonstrated impaired acquisition, reversal, and working memory in the Morris water maze (p<0.01). However these animals also manifested maintained pretraining hyperactivity and stereotypic behavior. The stereotypic behavior may interfere with the testing of memory in this case, and since lesions to the FF disrupt cholinergic and gabaergic input to the hippocampus these results may also have some relevance to stereotypy in human disease states with similar deficits.

415.8
RETROGRADE AMNESIA FOR PLACE AND CUE INFORMATION AFTER HIPPOCAMPAL DAMAGE IN RATS. M.P. Weisend* & R.J. Sutherland, Depts of Physiology and Psychology, University of New Mexico, Albuquerque, NM 87131.

Retrograde amnesia after hippocampal damage may share the same specificity as anterograde amnesia (Weisend & Sutherland, 1993). We report here a replication and extension of our previous findings. Seventy-four Long-Evans hooded rats were trained in the same pool on two versions of the Morris water task: 1) fixed, hidden platform (place task) and 2) visible platform discrimination (cue task). Rats received 80 trials with the hidden platform and 160 trials with the visible platform discrimination. Hippocampal lesions were made using microinjections of kainic acid. Ten rats served as controls, and 41 rats were lesioned. The lesioned rats were divided into 2 groups based on whether or not they were tested for place and cue task amnesia. Ten rats in each group were lesioned within 2 weeks. Retention testing was performed during the recovery phase, when the rats were retested to the platform and platform position. The rats were retested for place and cue task amnesia, and the lesioned rats showed deficits only on the place task, which is consistent with previous findings. Interestingly, rats that showed deficits on the place task but not the cue task showed deficits in a visible platform discrimination task, which is consistent with previous findings. These results suggest that the hippocampus may be involved in spatial memory, but not in cue memory.

415.10

Fimbria-fornix (FF) lesions in animals are frequently used to model neurodegenerative and traumatic neuropsychitragies observed in humans, and have been used to evaluate the therapeutic efficacy of a variety of interventions, such as grafting or pharmacotherapy. However, the recovery in the absence of interventions (i.e., spontaneous recovery) has rarely been investigated.

The present study was designed to assess spontaneous recovery of spatial learning and navigation in the Morris water maze (MWM) following bilateral kafoxic acid (KA) lesions of the FF. Subjects were 32 male Long-Evans rats. They were pretrained (6 days) or left naive and were either injected with KA or sham lesions. Post-training assessment in the MWM consisted of 3 days of training with the platform in one location followed by 3 days with the platform in the opposite quadrant of the maze. FF cuts produced a marked deficit in navigating to the first platform location. Pretraining attenuated the deficit. Considerable recovery was exhibited over the period of testing for the control group and for the pretrained group. Recovery was again observed which probe tests indicated entailed the use of a spatial strategy.

These results show that considerable spontaneous recovery does occur following bilateral transection of the FF but that recovery of spatial navigation may occur without recovery of spatial learning. Furthermore, these results suggest that a distinction may exist between interventions that enhance or speed spontaneous recovery and those that provide alternative recovery mechanisms. (Supported by NSERC Canada)

415.12

In order to study the involvement of the different components of the hippocampal formation in learning, rats with selective ibotenic acid lesions of the hippocampus, aspiration lesions of the entorhinal/parahippocampal cortex (EC/PFC), and controls were trained in Exp. 1 on spatial and nonspatial versions of an 8-arm Morris maze, and in Exp. 2 on concurrent visual discrimination and spatial rewarded alternation tasks. Rats with hippocampal lesions were especially impaired on the spatial tasks and did not differ from controls on the nonspatial tasks. In contrast, EC/PFC rats were like controls on the spatial tasks but were impaired on the nonspatial tasks. This pattern of results (e.g., a double dissociation) supports the view that the hippocampus and EC/PFC differ functionally. Specifically, the rats in the hippocampus seem to play an especially important role in the acquisition of spatial information, while the EC/PFC is involved in the processing of complex, nonspatial information.

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HIPOCCAMPAL AND NON-HIPOCCAMPAL BASED LEARNING IN THE RAT. Robert J. McDonald* and Norman M. White, Department of Psychology, McGill University, Montreal, Quebec, Canada.

Performance on a variety of place learning tasks is impaired by damage to the hippocampal system in the rat (Olton, Walker, & Gage, 1978; Sutherland, Kohl, & Whitehouse, 1993). The present experiment investigates the role of the lesion and the task environment in underlying place learning tasks that are not dependent on the hippocampal system is limited. The present experiment the participation in place learning of hypothesized memory systems that include hippocampal, parahippocampal and amygdala. The hippocampal system was studied by requiring rats to discriminate between 2 arms of an 8-arm radial maze. Two behavioral variables were manipulated: 1) Movement - rats were either confined to the arms of the maze for a fixed number of trials or were allowed to run in the maze freely; 2) Cue Resolution - the use of distal room cues was allowed by allowing the entire radia maze one arm position to the left at the start of each training day. Rats were discriminated between 2 arms of the maze, the first arm the rat could see a unique cue or set of cues, or between two adjacent arms from each of which they could see largely overlapping sets of cues. In the latter case the arms could be distinguished only by comparing the differences in the spatial relationship of each arm to many of the same cues. A series of single and double lesions revealed the following about the roles of the hippocampal and cue resolution variables in each of the three hypothesized memory systems. When the animals were confined to the arms of the maze only the amygdala system could process the information required to discriminate between the arms, and the discrimination was learned faster with unique than with overlapping cues. In both cases the passive discrimination was facilitated by hippocampal system lesions. When animals ran into the maze arms during training only the hippocampal system processed the information required to discriminate overlapping sets of cues, but both the hippocampal and dorsal striatal systems processed information that differentiated between unique cues. In the latter case the two systems appeared to process different kinds of information, in parallel, that led to the same solution.

RATS WITH ANGULAR BUNDLE TRANSCTIONS SHOW NORMAL ACQUISITION BUT IMPAIRED LONG-TERM RETENTION OF OBJECT DISCRIMINATIONS. V. Vogenic, J.C. Gibson, L.C. Kroemer, and L.A. Robinson, Department of Psychology, George Washington University, Washington, DC 20052, and 2nd Dept. of Cell Biology, Georgetown University, Washington, DC 20007.

Investigations of the neurobiology of memory using experimental animals have succeeded in modeling many of the characteristic features of amnesia seen in human clinical populations. To examine long-term memory, however, animal models of amnesia often employ extended measures of learning, with duration which is consistent to the retention measurements used with humans.

To determine the role of hippocampal/parahippocampal circuitry in both information acquisition and long-term retention, rats with bilateral transsections of the angular bundle were trained on three object discrimination problems and then retrained two weeks later to measure retention. Rats with discrete lesions of the angular bundle, which disrupt protractor function/(ontrol from entothial cortex and effector hippocampal-cortical projections, acquired the discrimination problems normally but showed a marked deficit in retention. Thus, the role of this circuitry may be limited to maintaining some types of information (e.g., single object discriminations) for retention. Behavioral paradigms that include a measure of retention may be particularly important for characterizing mnemonic deficits in animal models of amnesia. Supported by CNR and GWU Fundings.

DISOCIATION OF HIPOCCAMPAL AND STRIATAL CONTRIBUTIONS TO PLACE NAVIGATION IN THE WATER MAZE. B.D. Devlin*, E.H. Gould, L.H. Prin, Dept. of Psychology, Towson State University, Towson, MD 21204.

Previous studies have shown that damage to either the hippocampus or striatum of the rat results in severe spatial impairments. The purpose of the present study was to analyze the effects of hippocampal (fornix/limbic) and striatal (caudate-potamus) lesions on acquisition of the standard place version of the Morris water task as modified cue version that ascertained simultaneous acquisition of a place response. Rats with fornix/limbic lesions were impaired during a later stage of place task acquisition than rats impaired on the cue task. Caudate-potamus lesions resulted in a severe place task impairment and a transient cue task impairment, both of which were characterized by an early phase of thigmotactic swimming. The escape latency measure was particularly sensitive to thigmotactic behavior and not accurately reflect allocentric spatial learning. Post-hoc analysis of start point heading angles suggested that rats with fornix/limbic lesions used position response and guidance strategies. These rats demonstrated the same spatial bias for the former training quadrant on the platform removal probe and reduced flexibility in navigating to a novel platform location on the platform relocation task. In contrast, rats with caudate-potamus lesions spent a greater amount of time swimming in the former training quadrant on the platform removal probe, and despite poor place acquisition lacies showed greater improvement when required to learn a new platform position on the relocation task.

The results revealed dissociable aspects of water maze performance following damage to the fornix/limbic or caudate-potamus. It is suggested that the hippocampus mediates the allocentric spatial aspects of the water maze place task while the striatum may mediate acquisition of the non-allocentric procedural components of both place and cue versions. The results support a multiple neural-systems view of learning/memory and further suggest that is the intact mammalian brain multiple systems may acquire different forms of information simultaneously.

LESIONS TO THE HIPOCCAMPAL SYSTEM DO NOT IMPAIR SELECTIVE ASSOCIATIONS IN RATS. R.A. Murphy, R.J. McDonald, E.A. Germain & A.G. Baker, Department of Psychology, McGill University, Montreal, Quebec, Canada, H3A 1B1.

There is some evidence that lesions to the hippocampus impair performance on Kamin's blocking, on overshadowing and on a reversal procedure. However, these selective association procedures require subjects that ignore, to some extent, the relationship between a redundant CS and a US. It has been suggested that the hippocampus may be required for this type of selection. The present study compares rats with either electrolytic lesions of the fornix, or colchicine lesions of the dentate gyrus of the hippocampus, and controls on an appetizer operant version of Wagner, Logan, Haberland and Pisc's (1970) (US: Psy, 10' 171-190) procedure. We used a variable interval discrete-trial bar press procedure with two stimulus compounds containing a common element (XX, IX). Half of each group were trained with a True-discrimination (TD; AN: IX) in the first half of each session, while the other half were trained with a Pseudo-discrimination (PD; AX: BI: IX) in the second half of each session, so that both AX and BX trials were reinforced 50% of the time. In spite of the fact that X is reinforced equally often in both groups, previous research has demonstrated that in comparison to PD trained animals, TD trained normals show less responding to the partially reinforced stimulus (X) because the other more valid predictors of reinforcement (A and B) overshadow conditioning to X. During our test of X, all groups showed overshadowing of X following true-discrimination training. Consistent with previous experiments in our lab the lesion returns increased responding to X, protecting the reinforcement of AX and BX trials and during the test of X. These results suggest that the hippocampus is not required for this selective association phenomenon.

MOVEMENT-PRODUCED HIPOCCAMPAL AND PASSIVE NONHIPOCCAMPAL LEARNING IMPEDIE AMYGDALA-BASED STIMULUS-REWARD LEARNING. Norman M. White* and Robert J. McDonald, Psychology, McGill University, 1205 Dr. Penfield Ave, Montreal, Quebec, HSA 1B1.

We previously reported that the conditioned preference (CPP) learned by rats on the 8-arm radial maze using only distal cues to distinguish between food and no-food arms is eliminated by laminar nucleus of the amygdala lesions and potentiated by fornix lesions, suggesting that this form of stimulus-reward learning is mediated by a neural system that includes the amygdala but not the hippocampus, and that the hippocampal system interferes with amygdala-based learning of the CPP. 1) We tested the hypothesis that the cause of this interference is the acquisition of a spatial map of the maze route by the hippocampal system during 10 min of free-retrieval pre-exposure to the maze, given to all animals before CPP training. Rats not pre-exposed to the maze, or pre-exposed to a maze in a different room exhibited potentiated CPPs, comparable to those of animals with fornix lesions, suggesting that the CPP in normal animals is inhibited due to learning about spatial cues in the maze room. 2) Rats with fornix lesions made after pre-exposure (in the same room), or after CPP training but before testing, were prevented from obtaining the CPP compared to those observed in normal animals, suggesting that acquisition of spatial information by the hippocampal system during pre-exposure is required for a intact fornix; however, the fornix is not required for subsequent inhibition of stimulus-reward learning in the amygdala system. This inhibition may be mediated via entothial cortex. 3) When rats were pre-exposed to the maze by confining them in the arms (instead of exploring freely), the subsequently acquired CPP was comparable to that in normal animals, but was not potentiated by fornix lesions, suggesting that passive pre-exposure may produce non-hippocampal-learning, possibly mediated in the amygdala system, that impedes stimulus-reward acquisition by the same system. There may be two different latent-inhibition-like effects, involving two different kinds of learning and mediated by two different memory systems.


The historical debate between psychologists who favored "cognitive" learning theories and those who favored "stimulus-response" learning theories can be characterized by the tendency of rats to display place or response learning in the cross-maze paradigm selectively. Early studies suggested that either mechanism in learning to approach the consistently baited arm of a cross-maze (cf. Rente, 1957), raises the possibility that these two learning mechanisms are mediated by distinct neural systems. Consistent with this view, evidence indicating that the mammalian brain contains multiple memory systems which differ in the "type" of memory they encode. The present study was designed to examine the hypothesis that place and response learning are mediated by independent hippocampal and caudate nucleus memory systems, respectively.

Rats were trained to approach a consistently baited arm in a cross-maze from the start box (4 trials/day/14 days). On days 8 and 16 a single probe trial was given, in which rats were placed in the start box of the cross-maze opposite that used in training, and allowed to approach a maze arm. On the probe trials, rats which selected the baited maze arm were designated place learners, and rats which selected the un baited maze arm were designated response learners. 3 minutes prior to the probe trials, rats received bilateral hippocampus injections (solution A) (in order to produce neuronal inactivation) into either dorsal hippocampus or dorsolateral caudate nucleus. Saline treated rats received bilateral hippocampus injections on the day 8 probe trial, and response learning on the day 16 probe trial, indicating that with early training, there is a shift in learning mechanisms. L-Dopa injections into hippocampus showed no preference for place or response learning on the day 8 probe trial, and displayed response learning on the day 16 probe trial, indicating a blockade of place learning following injection. Rats receiving lidoacine injections into the caudate nucleus displayed place learning on both the day 8 probe trial and the day 16 probe trial, indicating a blockade of response learning following injection of the caudate nucleus. The results indicate that the hippocampus and caudate nucleus contribute to different place learning tendencies, respectively, and offer a "neurochemical" solution to the place versus response learning debate.

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A very extreme deficit in performance of the Morris Maze (MM) task was produced by small electrolytic lesions in rostral globus palidus (rGP, but not by lesions in the center of GP (Meyer & Cooper, Soc. Neurosci. Abst., 18:1565). The present study examined the nature of this deficit by testing performance in both negatively (MM) and positively (cheeseboard) reinforced tasks, and whether comparable deficits would be produced by biotenate lesions of rGP.

Testing in the MM began 4 weeks following surgery with eight 4-trial sessions of escape to a platform in the center of the same quadrant of a 1.6-m diameter tank. The platform was submerged after the first session, and further hidden by adding milk after the 2nd session. Two weeks later, the rats were tested during six 4-trial sessions to find half a Foxo Loop in the same spatial location amongst 177 holes. Rats with electrolytic lesions of the rGP failed to escape in the MM within 120 sec in most cases (5 out of 8 rats, and showed almost no reduction in their 60+ sec latency to find the food reward. By contrast, sham surgery and vehicle injection control groups, and also a group receiving 3 μg biotenate under ketamine anesthesia, achieved MM escape within 20 sec by the third session, and reduced their food finding latency from 60 sec to the first session to less than 20 sec by the fifth session. The experimental group receiving biotenate lesions of rGP under Nembutal anesthesia did exhibit deficient MM performance over the first four sessions, which might reflect a spatial navigation deficit. However, the more extreme deficits produced by electrolytic lesions in rGP suggest a broad disturbance of instrumental performance.

415.21 THE VENTRAL STRIATAL - VENTRAL PALIDAL AXIS AND ASSESSMENT OF EFFORT REQUIRED FOR A REWARD. V.J. Brown*, G. Symons, P. Wiss and M.P. Latimer School of Psychol., Univ. St Andrews, Fife, Scotland KY16 9JU.

The ventral striatum has been implicated in reward-related processing, but its separate contributions to the perception of reward value and reward cost is not known. The present study sought to determine the involvement of the ventral striatal-ventral pallidal (VS-VP) system in assessment of reward cost. We measured progressive ratio (PR) responding, in which the cost in lever presses of a single food pellet increases incrementally with each pellet. Bilateral ibotenate (IBO) lesions were made in VS (0.6, 0.8 μl injections/hemisphere; sham lesions 0.6 μl PFS) or VP (0.6, 0.5 μl injections/hemisphere; sham lesions 0.5 μl PFS) of rats after 21 days training and at least 30 days before testing started. The point when normal rats and sham controls stopped responding (breaking point) under a PR schedule was stable over days, as were latencies to collect pellets, the post-reinforcement pause increased as a function of schedule progression. These effects are consistent with previous reports. Rats with VS IBO lesions showed significant increases in breaking point, indicating a willingness to work at greater cost for pellets. Rats with VP IBO lesions also showed increases in breaking point, and a greater range of breaking points than controls. Rats with VP lesions did not show an increase in post-reinforcement pause as a function of the PR increments. As this increase reflects a reluctance to resume work in the light of perceived greater effort required for reward this result is interpreted in terms of a deficit in perception of increasing reward cost. Overall these data demonstrate that the effect of lesions in the VS-VP axis is not simply to decrease motivation to work for food reward. Rather, these data suggest that lesioned rats may have lost the ability accurately to perceive the increasing cost of reward. Thus, rather than a change in motivation per se there appears to be alteration in the perception of the increasing cost of reward.

416.1 CHANGES IN GLUTAMATE RECEPTOR PROPERTIES IN THE CEREBELLAR CORTEX OF CLASSICALLY CONDITIONED RATS. Sarah Pollock, Dave Layond*, Richard F. Thompson, Matti Mintz, & Georges Tocco. Psychobiology Unit, Psychology Department, Tel Aviv University, Israel & University of Southern California, Neurosciences Program, HEDIUC Neurosciences Building, Los Angeles, CA, 90089-2520.

The cerebellar cortex is believed to be involved in the neural plasticity associated with the conditioned eyelid response. Because glutamate receptors have been shown to play a pivotal role in various forms of synaptic plasticity, we planned to determine their involvement in this learning model using quantitative ligand binding autoradiography.

Male Wistar rats were trained on a delay paradigm with paired presentations of conditioned (tone) and unconditioned (electric shock to one eye) stimuli. Naive and unpaired animals were used as controls. Quantitative ligand binding autoradiography using ligands specific for the AMPA subclass of glutamate receptors revealed an increase in tritiated AMPA and CNQX binding on the contralateral side of the trained animals (compared to the ipsilateral) with little or no side difference on the unpaired and naive animals. Conditioning did not result in any change on tritiated TCP binding (NMDDA specific).

These results support molecular changes of glutamate receptors in cerebellar cortex correlated with learning.


Microinjections of a GABA_A antagonist picROTOXIN (PTX) in the cerebellum have been shown to block the expression of classically conditioned eyelid blink response. In the present study, we examined the effect of PTX on the acquisition of conditioned eyelid blink response. New Zealand white rabbits, implanted with cannulae aimed at the ipsilateral interpositus (IP) nucleus, underwent five days of standard delay tone-airpuff training while receiving continuous infusions (0.13 μl/min) of PTX (10 nmol/μl) into the IP. Then, they were given 3 days of training with artificial cerebrospinal fluid (ACSF) infusions. During the 5 days with PTX, animals showed no eyelid blink responses and normal unconditioned responses to the airpuff. When switched to the ACSF, these animals learned eyelid blink CRs as if they were naive. When PTX was infused again, CRs were completely abolished. These results support earlier reversible inactivation studies that demonstrated that the cerebellum is critical for acquisition and expression of eyeblink conditioning. Supported by grants from NARS 1F32MN010521-01 BNR to JK and NSF CNS-8178300 & NIH (NIA) AG05143 to RFT.
416.3


Metabotropic glutamate receptors (mGluRs) are known to play a crucial role in both the induction and maintenance of hippocampal long-term potentiation (LTP). It is conclusive, if LTP is of physiological relevance, that mGluRs should play an important role in functional long-term potentiation. As we investigated this hypothesis applying electrophysiological and behavioral standard procedures.

Electrodes were implanted into the perforant path for stimulation and in the dentate gyrus for recording in the rat. (R)-5-methyl-4-carboxyphenylglycine (ADCC), a selective mGluR2 antagonist, was injected in two concentrations (A=20.8 mg; B=104.2 µg i.c.v.) 30 min prior to tetanus-induced LTP or learning, respectively. For comparison, a novel form of shock-reinforced 4A,5aza-purine spatial alternation and a brightness discrimination task were performed. MCPG blocked different phases of LTP in a concentration dependent manner. Dosis B abolished a potentiation completely and also inhibited learning in the spatial task. These findings substantiate that mGluRs are involved in the expression of LTP in the dentate gyrus and consolidation of spatial alternation memory. The effects on both were dose-dependent, i.e. only the high concentration of MCPG induced a complete block, whereas no learning impairment occurred in the low dose group where the potentiation lasted up to 2.5 hours. We therefore assume, that STP in the hippocampus is the crucial factor for the learning of spatial tasks in the long term. Block of STP, however, results in amnesia.

416.5

THE EFFECTS OF MUSCIMOL INFUSIONS INTO THE AMYGDALA AND MEDIAL SEPTAL AREA IN MEMORY FOR MAGNITUDE OF FOOD REWARD. J.M. Williams, R.J. Nelson* and D.S. Olton, Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

Rats were trained on a go, no-go task measuring magnitude for food reward. In the study phase of the task, the rats were given one of two cereals. One cereal contained 25% sugar, the other 50% sugar. One of the two cereals was designated as the positive stimulus and the other as the negative stimulus. If the rat received the positive stimulus in the study phase, in the subsequent test phase the rat received another food reward which was placed beneath an object in a foodwell. Each rat received the positive and the negative food stimuli during the study phase, no food was placed beneath the object in the foodwell. Performance was measured as latency to uncover the foodwell in the test phase. A previous study using the aforementioned procedure revealed that lesions in the amygdala, but not the hippocampus significantly impair performance in this memory task. The present study seeks to further clarify the role of the amygdala in memory for magnitude of food reward. After achieving criterion (significant differences in latency to respond to the positive vs. negative stimulus), rats received cannula implantsations into both the medial septal area and amygdala. All rats received infusions into both of these areas and into the hippocampus. The effects of muscimol into each of these areas were compared to saline infusion trials and trials in which no infusions were given. The effects of muscimol on memory for magnitude of reinforcement will be discussed.

416.6

INTRA-SEPTAL INFUSIONS OF MUSCIMOL IMPAIR SPONTANEOUS AltTERATION PERFORMANCE: A FAILURE TO REVIVE LEARNING IMPAIROT WITH GlUCOSE M.B. Parent* and P.E. Gold. Department of Psychology, University of Virginia, Charlottesville. Virginia 22903.

Intra-septal infusions of drugs that act at cholinergic, noradrenergic, opioid peptidergic and glutamatergic receptors influence spontaneous alternation performance. The present experiment examined the role of medial septal GABAergic system on spontaneous defensive behavioral alternation performance by infusing the GABA agonist muscimol 15 min prior to training in a spontaneous alternation task. With evidence that systemic infusion of GABA antagonists reverse the impairing effects of intra-septal infusions of opioid agonists (Ragazzino, Parker, & Gold, Brain Research, 1982), the efficacy of intra-septal infusions of glucose in reversing long latency septal lesions to impair performance was assessed. The intra-septal infusions of muscimol (1 or 3 mmol. 0.5 µl/min) significantly impaired spontaneous alternation performance. Glucose (0.3, 1.6, 3.3 or 5.9 mmol, 0.5 µl/min) did not affect spontaneous alternation performance or reverse the deficit produced by intra-septal infusions of muscimol (1 nm). These results indicate that activation of GABAergic receptors in the medial septal impairs spontaneous alternation performance. This finding is similar to results obtained with morphine infusions. However, in contrast to the results with morphine, glucose did not reverse the muscimol-induced impairment. This pharmacological distinction suggests that glucose may interact with specific neurotransmitter systems in the medial septum to influence spontaneous alternation performance. Supported by NIA (AG 07648), NSF (BSR 9012239), and NIH (HD 07323).

Evidence for different types of memory in rats may lead to development of animal models for human memory disorders and provides information on neurobiological systems underlying these processes. The effects of the noncompetitive NMDA receptor antagonist MK-801 were investigated in those of the muscimol antagonist scopolamine hydrobromide on working and reference memory in the radial maze. In order to discriminate between the working memory and the reference memory, we used a modified version of the radial maze with the additional feature of a reward as the only criterion. Scopolamine (0.3 mg/kg, IP) or MK-801 (0.1 mg/kg, IP) were injected s.c. 30 min before a session. The duration of the session was 20 min, which was divided into 5 sub-sessions of 4 min each. Working memory was tested in the first sub-session by giving a reward in the arm of the maze, whereas the arm of the second sub-session, in which there was no reward, was used for testing the reference memory. The results showed that MK-801 impaired the ability to learn the reference memory, whereas scopolamine impaired the ability to learn the working memory. These findings indicate that MK-801 interacts with memory processes of similar complexity than does scopolamine hydrobromide, whereas the use of "difficult" and "easy" baiting patterns may be a sensitive method to analyze amnesic compounds.


The benzoazepine inverse agonist FG 7142 (20 mg/kg, i.p.) has been shown to selectively increase the DOPAC/DA ratio in the prefrontal cortex (PFC) of the rat. The effects of FG 7142 on the working memory functions of the PFC have not been examined. The present study tested the effects of FG on spatial working memory in the rat (delayed alternation in a T-maze) and the monkey (delayed response in a WGT). In rats, FG (20 mg/kg, i.p.) significantly impaired delayed alternation performance. This impairment was reversed by pretreatment with the benzodiazepine antagonist Ro 15-1788 (20 mg/kg, i.p.). Consistent with the hypothesis that FG impairs delayed alternation by increasing DA release, low doses of the DA antagonist, haloperidol (0.1-0.2 mg/kg, i.p.) were able to ameliorate FG-induced delayed alternation deficit. The glycinic partial agonist, (+)-HA-966 can act as an antagonist at the glycine site of the NMDA receptor. Pretreatment with (+)-HA-966 (10 mg/kg, i.p.) eliminated the effect of FG on delayed alternation. Similar responses were observed in monkeys: FG (0.2 mg/kg, i.m.) impaired delayed response performance, and this impairment could be reversed by haloperidol (0.005 mg/kg, i.m.) or the D1 antagonist SCH 23390 (0.005 mg/kg, i.m.). Preliminary results indicate that pretreatment with (+)-HA may attenuate FG-induced impairment. These results suggest that excessive DA release in the PFC impairs spatial working memory performance. (Supported by grants NSFP.9253354; MH 44866; and MH 14092.)

ANTEROGRADE AMNESTIC AND ANTICONVULSANT EFFECTS OF TWO TYPES OF NMDA RECEPTOR ANTAGONISTS: MK-801 AND HA-966. K. Xu*, P. Klíner, and R. Davis. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48104-1667.

The anterograde amnestic effects of noncompetitive NMDA antagonists MK-801 and HA-966 on classic fear conditioning in goldfish (Carassius auratus) were examined in a series of experiments. The results showed that MK-801 and HA-966, but not HA-966, impaired the acquisition of the classical fear memory. Analysis of dose-effect relationships revealed that MK-801 was more potent than HA-966 in producing anterograde amnesia, while HA-966 did not produce anterograde amnesia at the doses tested. These findings suggest that MK-801, but not HA-966, produced anterograde amnesia by their antagonism of NMDA receptor complex specifically.


We evaluated the effects of the GABA antagonist baclofen on learning and performance of rats in a water maze task. Male Long-Evans rats were trained to a criterion of threefold above chance level of performance in the maze. Once rats achieved criterion, (-)baclofen (1 mg/kg) was injected i.p. in half of the rats 15 min prior to testing on each of 3 consecutive days. The treated rats took significantly longer to complete the maze. In two additional studies, we gave the rats daily injections of (+)baclofen (1 mg/kg) prior to training them on new configurations of the maze. We found no significant difference between the treated and control rats on the number of trials required to reach criterion but did find that after reaching criterion the baclofen-treated rats required significantly longer times to complete the maze. When no drugs was administered, the performance of the two groups was not significantly different. These results suggest that baclofen impairs performance but does not affect learning.

SPERMIDINE POTENTIATES DIZOCILPINE-INDUCED LEARNING IMPAIRMENT OF RATS IN A 14-UNIT T-MAZE TO DEMONSTRATE POLYAMINE MODULATION OF NMDA RECEPTOR FUNCTION. R. J. Spanier, E. F. Hepler, and R. L. Ingram. Gerontology Research Center, NIA, and Addiction Research Center, NIDA, NH, Baltimore, MD 21224.

The NMDA receptor, a membrane ion channel complex has been reported to be involved in memory processes. Learning is impaired following administration of dizocilpine (DIZO), a non-competitive antagonist of the NMDA receptor. Polymers of spermidine, a polyamine, interact with the NMDA receptor to enhance binding of DIZO, which blocks the ion channel. The present study assessed action of polymers of spermidine as releasing agents for the NMDA receptor activation. DIZO (0.05 mg/kg) was given i.p. before maze learning, at a dose that produced a slight, non-significant reduction in performances (t = 0.5, p < 0.05). Administration of spermidine increased the response of rats to DIZO. The results confirm that systemic injection of a polymer can modulate learning processes involving the NMDA receptor. Studies are currently being undertaken to test the hypothesis that systematically injected SPD may ameliorate impaired maze learning in aged rats by activating the NMDA receptor.

SELECTIVE ENHANCEMENT OF AMPA RECEPTORS IMPROVES NEO-CORTICAL LEARNING. R. Graugler*, C. Manjouze, A. Angelen, A. Lopez, M. Davis, B. Tran, G. Rogers & G. Lynch. CNIM, Univ. of Calif., Irvine, CA 92717.

In a novel neocortically dependent auditory task, the first pharmacological agents to specifically enhance the excitatory activity of AMPA receptors in the brain ("AMPAKINE") were tested for their effects on learning. AMPAKINE selectively enhance the glutamate receptor, which mediates all normal fast excitatory transmission, and is the likely site of synaptic long-term potentiation (LTP), the leading candidate substrate for learning. In the first reported tests of behavioral effects of these drugs, Stabili et al. (Proc. R. Soc. B, 1994) and Graugler et al., (Nature, 1. 328:1993) showed that they enhanced learning in a radial arm maze and an olfactory discrimination task, which are hippocampally and neocortically dependent, respectively. We report here on a novel auditory discrimination learning task, and show that performance on this task is improved by AMPAKINE. The task consists of a maze in which each arm has a high-frequency speaker. Only two speakers are active on any given trial. Each speaker continuously plays a complex natural sound, recorded and then shifted up into the rat's learning range (10kHz and above). On each trial, the rat receives a water reward for approaching the "correct" one of the two sounds, whose spatial location changes with each trial. Rats learn many such sounds rapidly, and exhibit weeks-long retention of learned sounds. Selective lesions of primary auditory neocortex severely impairs learning of new sounds. In an experiment counterbalanced for sound preference, learning and retention, and in which animals serve as their own controls for drug effects, rats were tested on very difficult sound discriminations, and a small number of training trials. On days when they were not given the drug, animals were unable to learn the sounds significantly above chance acquisition levels. On days when they received the drug before entering the maze, they exhibited a 41% improvement over their non drug performance. This is the first demonstration of enhancement of learning in a neocortically dependent animal model. (Supported by AFOSR F49620-92-J-0037 and ONR N00014-93-J-1205.)
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416.15  
A CRITICAL ROLE FOR THE NMDA RECEPTOR SITE IN COCAINE DRUG CONSUMPTION PROCESSES. Ernest N. Damianopoulos* and Robert J. Carey. Psychiatry, SUNY Health Science Center and Research and Development Service-151, VA Medical Center, Syracuse, NY 13210.  
The role of the NMDA receptors in cocaine conditioning and sensitization was studied in four groups of matched Sprague-Dawley rats. A sub-motoric dose of the NMDA antagonist MK-801 (0.1 mg/kg ip) was employed using a novel drug-compartment Pavlovian drug conditioning paradigm. The animals were placed sequentially in two similar but distinct test environments. In the first compartment, the animals always received a non-drug test (20 min). Upon removal, the animals received either saline, cocaine (mg/kg ip), MK-801 or MK-801 plus cocaine depending on group assignment and were then placed immediately into the second compartment (20 min). Every other day over a 12-day period, the animals of each group (n = 5) received a non-drug test followed by 1 saline/drug test. Across all drug treatment days and subsequent tests for conditioning, there were no statistical differences between the saline and drug treatment groups in the non-drug test environment on locomotor distance. Thus, non-specific, non-associative response sensitization effects were not observed. Cocaine, however, did have a consistent stimulant effect on locomotor behavior in the drug test environment either when administered alone or in combination with MK-801. Following a 1-day and again after a 21-day withdrawal, all animals were administered a non-drug test for conditioning. The results showed that the cocaine-induced enhancement of locomotion was conditioned to the aversive cues of the drug-associated environment but this effect was not present in the animals treated with cocaine plus MK-801. While MK-801 blocked cocaine conditioning, MK-801 had no effect on the cocaine locomotor stimulant response.  

416.16  
PREGNENOLONE SULFATE INFUSION ANTAGONIZES DIZOCILPINE AMNESIA: MEDIATION BY ALLOPREGNANOLONE. D. L. Cheney*, D. Uzunov, E. Costa and A. Guidotti. FGIn, Georgetown University Medical Center, 3900 Reservoir Road, N.W., Washington, D.C.  
Dizocilpine, a non-competitive N-methyl-D-aspartate (NMDA) antagonist (0.1mg/kg, i.p.), disrupted the passive avoidance response in control and adrenalectomized/castrated (ADX/CX) male rats. Infusion of pregnenolone sulfate (20mg/kg i.v., 5min) antagonized dizocilpine induced amnesia and increased the brain content of pregnenolone, progesterone and allopregnanolone as measured by gas chromatography-mass fragmentography. Similar effects were observed with CPPene, a competitive NMDA antagonist (2.5mg/kg, i.p.). Five to ten fold increases in allopregnanolone were observed in olfactory bulb, striatum, hippocampus and cortex following infusion of pregnenolone sulfate. Pretreatment with the 5a-reductase inhibitor SKF105111 (5mg/kg, i.v.) blocked the pregnenolone sulfate antagonism of dizocilpine amnesia and prevented the increase of allopregnanolone in olfactory bulb, striatum and cortex. These results support the hypothesis that the amnesia elicited by the NMDA antagonist dizocilpine is counteracted by pregnenolone sulfate via its ability to increase the brain content of allopregnanolone.  

416.17  
EFFECTS OF PHENYTOIN ON LEARNED APPETITIVE RESPONSSES AND THE SUBSEQUENT ACQUISITION OF ESCAPE AND AVOIDANCE RESPONSES. N. L. Mohr1, J. E. Steinmetz2*, and P. F. Garraghty1, 2. Dept. of Psych. and Prog. in Neural Sci., Indiana Univ., Bloomington, IN 47405.  
Phenytoin (Dilantin) has been used for the control of epileptic seizures for more than 55 years. This drug has proven to be an effective antiepileptic, but it may also be associated with deficits in behavior and cognition. Here, we have evaluated the effects of instituting chronic phenytoin maintenance in rats that had undergone initial training in an appetitive instrumental task with a subsequent switch to an aversive (escape/avoidance) task. We initially trained deprived rats to press a bar in the presence of a tone stimulus for a food reward. We then began treating the rats daily with phenytoin (solution, Parke-Davis). Our goals were to determine the extent to which the animals' behavior in the appetitive task was affected by the institution of phenytoin treatment, and how the acquisition of the new escape/avoidance responses were affected. Our results suggest three findings relative to control observations from drug-free rats: 1) a reduction in response latency in the appetitive phase of the experiment after the initiation of drug treatment; 2) a prolonged latency (over session days) in the acquisition of the initial escape response after the animals are switched to the aversive task; and 3) a failure to acquire the avoidance response. These results, in turn, suggest that phenytoin has substantial, and negative (at least for findings 2 and 3) consequences for learning in subjects chronically maintained on the drug, perhaps because of its antagonistic effects on NMDA receptors.

417.1  
ACUTE DIAZEPAM ADMINISTRATION PRODUCES MEMORY DEFICITS SIMILAR TO THOSE DUE TO CHRONIC ALCOHOL CONSUMPTION. N. Berde, R. Jaffard and D. Bercacoeur*. Lab. Neurosciences Comportementales et Cognitives, CNRS URA 339, Univ. Bordeaux I, avenue des Facultés 33405 Talence Cedex, France.  
The effects of the benzodiazepine agonist (Diazepam) on delayed alternation in a T-maze as mice were studied. Delay intervals (DI) separating the acquisition trials from the retention trial were either 30 sec or 6 hours. The 6 hours trial was further used as an acquisition trial for a subsequent alternation trial (DI: 30 sec) aimed at measuring short-term memory as well as the eventual sedative effects of the drug. Diazepam (1.0 or 1.5 mg/kg) was administered 1.50 min before the 6 hr retention trial. Results showed that the 1.5 but not the 1.0 mg/kg dose induced a memory deficit, as compared to saline treated subjects. No impairments were observed on the short-term trial. Furthermore, the Diazepam-induced memory impairment was reversed by a context-change occurring before the retention trial even though this finding requires further experiments to be firmly established. The effects of Diazepam on memory appear to be similar to those resulting from a long-term ethanol administration and which appear to result from an impairment of the retrieval phase of memory processes.  
Supported by CNRS URA 339.

417.2  
The functional activity of cholinergic neurons in the medial septum (MS) that project to the hippocampus is modulated by an intrinsic GABA-benzodiazepine (BDZ) mechanism. Intraseptal injection of the BDZ, chlordiazepoxide (CDP) to rats (i) impairs working memory (WM), (ii) decreases hippocampal high-affinity choline transport, and (iii) decreases dentate gyrus extracellular responses. Further, intraseptal flumazenil, a BDZ antagonist, reverses systemic CDP-dependent amnesia (Stackman & Walsh, 1992; Walsh et al., 1993; Stackman et al., 1993). These data indicate a septal BDZ substrate capable of impairing WM and modulating cholinergic activity in the HPC, is likely the site of action of the amnestic action of CDP. Experiment 1 examined the contribution of neural regions proximal to the septohippocampal network, to the expression of CDP-induced amnesia. Infusion of 30 nmoles of CDP into the MS impaired WM in a delayed non-match-to-sample (DNMTS) radial-arm maze (RAM) task. This dose of CDP failed to alter RAM performance when infused unilaterally into the cingulate gyrus, or bilaterally into the lateral septum, or the nucleus basalis. Infusion of the local anesthetic, lidocaine (147 nmoles) into these sites produced similar behavioral effects. Experiment 2 examined the time-dependent nature of intraseptal CDP-induced amnesia. Rats were trained in the DNMTS RAM task and then injected with 30 nmoles CDP into the MS at one of the following postraining times: 0, 15, 30, 45, or 60 mins. Rats infused with CDP immediately postraining exhibited WM impairment. Infusion of CDP at the other postraining times failed to significantly impair performance. These data indicate that MS is the site of action for CDP-induced amnesia, and this structure is critically involved in an early encoding or maintenance phase of spatial WM. Supported by NSF Grant BNS 9222097 to JW.
417. 3  
CHRONIC BENZODIAZEPINE (BDZ) TREATMENT FACILITATES SPATIAL LEARNING IN THE MORRIS WATER MAZE (MWM).
M.S. Mohamed, W.S. Messer and E.I. Tzitz.  
1Dept. of Medical and Biological Chemistry, College of Pharmacy, University of Toledo, 43606, and 2Dept. of Pharmacy, Medical College of Ohio, Toledo, OH 43609.
Acute BDZ treatment, which potentiates GABA inhibition interferes with long-term potentiation (LTP) and impairs spatial learning. Reducing GABA inhibition in hippocampal CA1 region facilitates LTP. Chronic flurazepam (FZP) treatment reduces GABA inhibition in the CA1 region in vitro. We hypothesized facilitation of LTP in hippocampal slices from chronic FZP-treated rats. However, in a previous study using 10 theta bursts, LTP was induced to a similar degree in FZP-treated and control slices but was not maintained in FZP-treated slices over time. This study mediates spatial learning we evaluated the effect of 1 week FZP treatment (10 mg/kg x 3 days; 150 mg/kg x 4 days, p.o. to 0.2% saccharine) in the MWM. Treated rats were offered saccharine H2O for 2 days before and after FZP treatment. Control rats received saccharine H2O. Rats were adapted to the maze by allowing them to swim freely for 120 s over 2 days. Two days after FZP-treatment, we tested each rat’s ability to find a hidden platform. Rats were trained 4 trials/day/4 days and tracked with a video camera connected to a computer. Latency to escape(s), path length (cm), and speed (cm/s) were averaged. Speed was constant for each group (p > .05) indicating no motor effect due to FZP treatment. The latency of FZP-treated rats to find the platform was less than controls (treatment effect, p = .043) suggesting that reduced GABA inhibition associated with chronic BDZ treatment facilitates spatial learning. However, our previous hypothesis. Future studies will explore LTP in chronic BDZ-treated rats using a 5 theta burst paradigm. Supported by NS01493 to W.S.M. and NIDA grants ROI-DA04075 and R01-R01DA10838 to E.I.T.

417. 5  
EFFECTS OF CONVULSANT AND ANTICONVULSANT AGENTS ON MEMORY IN SQUIRREL MONKEYS. J.M. Moreschibecker* and E.D. Pelletier. Department of Pharmacology, LSU Medical Center, New Orleans, LA 70112.
It has been reported that subconvulsant doses of convulsant agents such as strychnine and pentylenetetrazole may enhance memory in rodents studied under various behavioral procedures. The present study was designed to determine if similar results might obtain in monkeys. Responding by squirrel monkeys was maintained by food presentation under a repeated acquisition of behavioral chains procedure. Monkeys acquired a different three-response chain each session. Sequence completions were reinforced under a fixed-ratio 5 schedule (FR 5) and errors produced a brief timeout. After the monkey reached a predetermined acquisition criterion, the session was stopped and either a 90 min or 24 hr delay was interpolated. Following the delay the subject was retested on the same discriminations and responses at quantitatively the same percent accuracy. When administered immediately after the monkey reached the acquisition criterion strychnine and pentylenetetrazole had no effect on percent savings under the 24 hr delay. Similarly, the delta opioid agonist, BWSW4536, 4-[(R)-2-(3,5-Dimethyl-1-piperazinyl)-N-N-dimethylaminoethylazidohydrochloride], had little or no effect on percent savings following either 90 min or 24 hr delays. This was true even at doses of BWS4536 which produced convulsions. In contrast, at high doses triazolam decreased percent savings following a 24 hr delay. These results suggest that at subconvulsant doses convulsant agonists have little or no effect while anti-convulsant agents such as triazolam can disrupt mnemonic processes in squirrel monkeys. (Supported by USPHS Grants DA05373 and DA04775.)

417. 7  
Using a recently developed, valid task for the measurement of sustained attention in rats (Buschini et al., 1994; McGeachy & Sarter, 1994), the benzodiazepine receptor (BDZ) agonist chlorziazepoxide (CDP) was found to potently and dose-dependently decrease the relative number of hits but not of correct rejections. Furthermore, the effects of CDP interacted with signal salience. As expected in intact animals, BDZ inverse agonists failed to facilitate performance. Here we show that intracerebroventricular (ICV) infusions of CDP (20, 40 μg/μl hemisphere) or B-CCM (1.5, 3.0 μg/0.5 μl hemisphere) into the substantia innominata of the basal forebrain reproduce the effects of the systemic administration of these compounds. Moreover, the effects of intrabasal CDP were more efficacious in decreasing the relative number of hits than of correct rejections. From the effects of systemic administration, these potent effects of intrabasal CDP on vigilance performance were not associated with equally potent effects on the number of errors of omission. These data support the hypotheses that the basal forebrain is a sufficient system for modulating the attentional effects of BDZ agonists. Also, these findings parallel the effects of systemic and intrabasal administrations of BDZ ligands on cortical acetylcholine efflux (Moore, Sarter & Brunero, 1993, 1994).

417. 4  
The effects of diazepam (0.2, 0.8, 1.6 and 3.2 mg/kg) on the primate and memory effects were evaluated in four groups of monkeys using a serial probe recognition (SPIR) task. Only the highest dose of diazepam (3.2 mg/kg) significantly disrupted the monkeys' performance on the SPIR task, causing an increase in the number of errors and an increase in the response latencies on the probe trials. Analyses of the serial position curves for each monkey further revealed that the highest dose (3.2 mg/kg) of diazepam disrupted both the recency effect and the middle portion of the serial position curves in all the monkeys tested. In contrast, the primacy effect was unaffected by any of the doses of diazepam tested. Previous reports have suggested that the primacy effect is mediated by the establishment of contextual or configural associations and the recency effect is mediated by the establishment of simple associations. Therefore, we have proposed that diazepam interferes with the simple associative processes involved in the recency effect to a much greater extent than it does with the contextual or configural associative processes involved in the primacy effect. This is the first demonstration showing that diazepam can produce such selective memory impairments in nonhuman primates.

417. 6  
Contingent drug tolerance is a widely reported phenomenon. However, the principles by which contingent tolerance develops are not well understood. Unlike other forms of drug tolerance, contingent tolerance does not appear to be under the influence of Pavlovian conditioning. The present study investigated the role that latent inhibition and partial reinforcement play in the development of contingent tolerance to the anticonvulsant effects of diazepam (DZ), in experiment 1, kindled rats were injected with either 5mg/kg of DZ or vehicle without convulsive stimulation, three times daily, for forty-eight hours following the last injection, both groups were switched to a DZ before stimulation schedule to assess the development of contingent tolerance to the anticonvulsant effects of 2mg/kg of DZ. Neither group displayed significant tolerance on the first trial, however, both groups developed contingent tolerance within 15 trials. Prior DZ exposure alone, not only, failed to retard, but facilitated the development of contingent tolerance. In the second experiment, kindled rats were exposed to a schedule which alternated between DZ injection followed by convulsive stimulation and DZ or vehicle injection following by handling. Partial pairing of DZ injections with convulsive stimulation (reinforcement) failed to retard the development of contingent tolerance to the anticonvulsant effects of 2.5 mg/kg of DZ.
Further, the extinction of tolerance in the two groups did not differ significantly. The results of these two experiments suggest that contingent tolerance to the anticonvulsant effects of DZ is not under the influence of latent inhibition or partial reinforcement.
MEMORY ENHANCEMENT WITH THE DELTA OPIOID RECEPTOR ANTAGONIST NALTIRINDOLE IS DEPENDENT ON TRAINING PARAMETERS

ELLEN B. BENNETT & M. R. ROSENZWEIG. Dept. of Psychology, Univ. of California, Berkeley, CA 94720.

Administration of naltirindole or delta opioid agonists either enhance or impair memory formation, these effects are dependent on treatment and training conditions. We examined whether memory effects can be produced by administration of the delta receptor antagonist naltirindole (NTI). Two-day-old chicks were injected with saline or NTI in the morning and trained 5 min before one-trial peck avoidance training. To give relatively weak training so that either impairment or enhancement could appear, chicks received either of 2 training conditions: (1) The Latent Inhibition (LI) condition involved 4 pretrained exposures to a dry chocolate bead followed by one exposure to a bead coated with the chemical aversant methylnaltrexone (MeA). (2) No-LI chicks were given one exposure to a bead coated with 1% MeA. Approximately 40-50% of saline injected chicks in each training condition avoid pecking a dry bead at 24 h test. In LI trained chicks, NTI caused a dose dependent increase in avoidance at 24 h test, the highest dose producing significant memory enhancement. In contrast, NTI had no significant effect in the No-LI trained chicks. These findings suggest that memory enhancement resulting from opioid antagonism is dependent on training conditions. Specifically, endogenous opioids may be selectively involved in memory formation for training that involves discrete information about conditioned stimuli.

Supported by NIDA DA04902 (PJC), DA04795 (MRB) & DA04195 (JLM).

MODULATION OF MEMORY FORMATION FOR A ONE-TRIAL PECK AVOIDANCE TASK IN CHICKS GIVEN CENTRAL INJECTIONS OF OPIOID RECEPTOR SELECTIVE OPIOID ANTAGONISTS. D.W. Lee*, E.L. Bennett & M.R. Rosenzweig. Dept, of Psychology, University of California, Berkeley, CA 94720.

To investigate opioid-mediated memory formation, chicks were injected with opioid antagonists or saline 5 min before training on a one-trial peck avoidance task, then tested 24 h later for retention. A weak level of training (10%) of the aversant liquid methylnaltrexone (MeA) was used as the aversive stimulus; 10% MeA results in intermediate retention levels (~40%) allowing the investigation of both memory impairment and enhancement within identical experimental protocols. Several experiments were conducted administering i.c. bilateral injections of either mu, delta, or kappa selective antagonists (CTOP, CTOP(0.01mM), norBNI, respectively) or saline directed into the region of the intermediate medial hyperstriatum ventrale (IMHV, the avian homolog of mammalian visual association cortex) or tubus parafacialis (LPD, homolog of basal ganglia).

Mu antagonists CTOP (01 nmol/hemisphere) impaired memory when injected into the LPD only. Delta antagonist ICI enhanced memory in both the IMHV (3.0 nmol/hemisphere) and LPD (10.0 nmol/hemisphere) but only in LPD revealed significant (p < 0.1). Kappa antagonist norBNI impaired and enhanced memory in a dose dependent way when injected into the IMHV (10.0 nmol/hemisphere impaired; 10.0 nmol/hemisphere enhanced). The same pattern was observed when norBNI was injected into LPD but only impairment by 30 nmol/hemisphere was found to approach significance (p < 0.1). Although opioids clearly modulate memory formation in the chick, the direction of their effects (impairment or enhancement) is highly dependent upon 1) dose; 2) receptor type (mu, delta, or kappa); and 3) brain location (IMHV or LPD).

Supported by NIDA grants DA05396 (DLC) and DA04795 (MRB).


Work in our laboratory has recently shown that acute opioid withdrawal elicited by naloxone is enhanced when morphine is administered in the presence of shock associated cues. To further explore the nature of these effects, two experiments were conducted to determine whether naloxone could elicit signs of opioid withdrawal in animals exposed to a context associated with shock even in the absence of morphine. Experiment 1, utilizing a high (10 mg/kg) dose of naloxone, revealed that forepaw tremors but no other classic opioid withdrawal signs could be induced by naloxone following exposure to a context associated with shock. Experiment 2 further examined this effect across a range of naloxone doses (0.1, 1, 10 mg/kg). This experiment revealed that the emergence of forepaw tremors following exposure to a shock context associated is dependent upon a high (10 mg/kg) dose of naloxone. Furthermore, the severity of withdrawal such as mastration and teeth chattering were most evident at lower doses of naloxone. These findings are consistent with other studies that have shown that naloxone can induce signs of opioid withdrawal following the exposure to physical stressors.

NALOXONE AUGMENTS PAVILIONIAN CONDITIONING OF CONCOMITANT HEART RATE AND MEDIAL PREFRONTAL CORTEX NEURONAL RESPONSES. L.L. Hernandez*, E.L. Watson, and C.J. Glaser. VA Medical Center and U South Carolina, Columbia, SC 29201.

Low doses of naloxone (0.1 mg/kg) augment Pavlilian cardiac conditioning and delay its extinction in rabbits. Since the medial prefrontal cortex (mPFC) participates in Pavlinian conditioning, we assessed whether SNX (0.1 or 0.5 mg/kg, i.v.) has parallel influences on conditioned heart rate responses (HR C) and concomitant, pretraining, multi-unit activity (MUA) during differential Pavlilian training (one tone Cs+ paired with shock, another Cs- presented alone). Prior to training, each CX dose reduced the magnitude of HR orienting responses to the tones but did not alter tone-evoked increases in PFCm MUA, compared to saline. During conditioning, 0.5 mg/kg increased the overall magnitude of HR CRs, while 0.1 mg/kg reduced HR CR magnitude; nonetheless, each dose enhanced HR discrimination during testing. Further, Cs+ evoked activity increased that grew larger during training and remained elevated. These effects were greater magnitude to the Cs+ vs. -Cs- amplified MUA discrimination during training and extinction, but the higher dose increased, while the lower decreased, the size of the MUA CR to the tone that HR has similar effects on CS-evoked changes in HR and PFCm MUA during Pavlilian training and extinction, and suggest that endogenous opioid systems in or afferent to the PFC modulate Pavlilian discrimination.

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417.15


Recent findings suggest that a modest increase in circulating glucose levels directly modulates brain mechanisms involved in mnemonic processing. Glucose infusions into the septal area reverse amnesia induced by morphine infusions. Amnesia following intraseptal morphine infusions may be due, in part, to opioid inhibition of cholinergic neurons with glucose reducing this effect. The present experiment determined whether intraseptal glucose reverses the effects of intraseptal morphine injections on acetylcholine (ACh) release in the hippocampal formation and on memory. Samples of extracellular ACh were assessed in two intervals using in vivo microdialysis with HPLC-EC. From 20-40 min after intraseptal morphine injections (4.0 nmol), ACh output was reduced by 25% compared to baseline levels. Concomitant treatment with glucose (18.3 nmol) blocked this effect, with ACh output similar to baseline rates. Glucose and CSF injections alone did not change ACh output compared to baseline. Two days after microdialysis testing, rats received septal infusions 25 min prior to spontaneous alternation testing. Intraseptal morphine infusions reduced alternation scores which were reversed by concurrent glucose infusions. The effects of intraseptal infusions on alternation performance and ACh output were significantly correlated. These findings suggest that glucose may ameliorate memory deficits produced by intraseptal morphine infusions by directly or indirectly blocking the opioid inhibition of cholinergic neurons. (Supported by NSF (BNS-9012239), NIA (AG 07848) and DNR (IN001489-J-1216)).

417.17

ENHANCEMENT OF MEMORY PROCESSING IN AN INHIBITORY AVOIDANCE AND RADIAL MAZE TASK BY POSTTRAINING INFUSION OF BOMBESIN INTO THE NTS. C.L. Williams* & J.L. McGaugh, Center for the Neurobio. of Learning & Memory and Dept. of Psychology, U. of Calif., Irvine, CA 92717-3800.

Bombesin is a peptide known to modulate memory storage when given either systemically or intraventricularly immediately after training. Two days after training it was not possible to determine whether the nucleus of the solitary tract (NTS) mediates the effects of bombesin on memory. In the first experiment male Sprague Dawley rats were trained in an inhibitory avoidance task (0.5 mg/kg of morphine) and bombesin or vehicle was infused unilaterally into the NTS through implanted cannulae immediately after training. Retention was assessed either 1 or 7 days later. Doses of bombesin ranging from 5 to 50 ng of bombesin significantly enhanced retention on the day test (p < .05 and .01 compared with vehicle controls respectively). There were no differences between the drug and control groups on the seven-day retention test. In the second experiment, bombesin (25, 50, or 250 mg) or vehicle was infused unilaterally into the NTS immediately after the animals were trained in a radial arm maze task. On retention tests given 18 hours later, groups that received 25 or 50 ng of bombesin made a significantly greater percentage of correct choices on the retention test than did the vehicle-treated controls (p < .02 and .05 respectively). The findings indicating that bombesin influences retention by activating the NTS is consistent with recent evidence suggesting that the NTS is involved in regulating memory storage.

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417.19

NEUROTENSIN RECEPTORS DECREASE AS A FUNCTION OF AGING AND COGNITIVE PERFORMANCE IN THE RAT BRAIN. J. Goldsworthy*, E. Khetani, M.J. McNeely & R. Quinlan, Dept. of Psychiatry, Neurology and Neurosurgery, Douglas Hospital, Res. Ctr, McGill Univ., Montreal, Canada, H4H 1R3

Cognitive deficits are often associated with the disease and dysfunction of cholinergic function. However, functional changes in the activity of other neurotransmitter systems such as neurotensin (NT) can also affect cognitive behavior. High concentrations of NT-like immunoreactivity and receptors are often found associated with areas known to be involved in mnemonic processing as well as cholinergic neurons. Further, alterations in NT mRNA density have been reported to be associated with the amnesia produced by lesions of basal forebrain neurons (Wenk et al., 1989, Behav. Brain Res., 33: 367-95). Thus, the objectives of the present study were to evaluate if NT-like immunoreactivity in the aged rat brain and if these alterations are related to cognitive status. Aged (24-25 month old) Evans rats were behaviorally evaluated using the Morris Water Maze task (3 trials/day for 5 days). Animals were classified as either aged, cognitively impaired (AI) or cognitively unimpaired (AU) based on their performance in the task compared to young rats (YR). NT-like immunoreactivity (NTL) binding was observed in the hippocampal formation (dentate gyrus), entorhinal cortex, amygdala, and hypothalamus as a function of age. Both aged groups also showed significant (p<.05) reductions in NT binding sites compared to CTX in the hippocampal CA3 sub-field, with the AI animals exhibiting the lowest levels. Thus, the alterations in [3H]NT binding may be a systematic involvement of NTergic systems in age-related cognitive deficits.

In the middle and central temporal area [3H]NT binding sites were decreased as a function of age while binding in the medial forebrain bundle was decreased as a function of age and cognitive status. These decreases in [3H]NT binding may be a result of a decrease in the NT levels and their receptors in a variety of brain areas (see Hertz et al., 1994, that meeting).

417.20

DOPAMINE D1 DRUGS MODULATE LEARNING ABILITIES OF AGED MEMORY IMPAIRED RATS. C.L. Moore*, S. Ede, J.J. Horst†, W. VPN, R. Chamoun* and P. Gaudette†, 1Douglas Hospital Res. Ctr. and Dept. of Neurology/Neurosurgery, McGill Univ., Verdun, Q. Canada H4H 1R3, 2Hospital Notre Dame, Dept. of Neurosurg., Universite. de Montreal, Q. Canada H2L 4R5

Lately, it has become apparent that besides acetylcholine, a number of other neurotransmitters contribute to learning and memory either directly or via their interaction with the central cholinergic system. For example one of the spatial mnemonic deficits brought on by pharmaceutical manipulations such as hippocampal deservation or the blockade of cholinergic receptors are reportedly attenuated by the administration of dopaminergic (DA) drugs. It is also known that DA drugs can facilitate learning in various paradigms in young animals. In the present study, we examined if such drugs could modulate learning in aged animals suffering from cognitive deficits. 24-25 month old Long Evans rats (3 trials/day for 5 days). Animals were classified as either aged, cognitively impaired (AI) or cognitively unimpaired (AU) based on their performance in the task compared to young rats (YR). NT-like immunoreactivity (NTL) binding was observed in the hippocampal formation (dentate gyrus), entorhinal cortex, amygdala, and hypothalamus as a function of age. Both aged groups also showed significant (p<.05) reductions in NT binding sites compared to CTX in the hippocampal CA3 sub-field, with the AI animals exhibiting the lowest levels. Thus, the alterations in [3H]NT binding may be a systematic involvement of NTergic systems in age-related cognitive deficits.

In the middle and central temporal area [3H]NT binding sites were decreased as a function of age while binding in the medial forebrain bundle was decreased as a function of age and cognitive status. These decreases in [3H]NT binding may be a result of a decrease in the NT levels and their receptors in a variety of brain areas (see Hertz et al., 1994, that meeting).
[ kurz text ]
418.5 DISHARMONY OF ACOUSTIC STARTLE SHOWS FREQUENCY CATEGORIZATION BY CRICKETS. R.L. Wyettbach* and R.R. Hipf, Neurobiology: Animal Behavior, Cornell University, Ithaca, NY 14853

Flying Polynesian field crickets (Teleogryllus oceanicus) have an acoustic startle response to pulses of ultrasound. This consists of several movements that steer the cricket away from the sound source. One aspect of the response, metathoracic leg swing, is of short latency and duration.

Ultrasound startle deceleration with repetition in a way consistent with standard cricket habituation. If deceleration is not elicited, recovery is rapid. With repetition, deceleration becomes slower, with a longer latency and a longer duration.

We have used this test to determine which frequencies of sound are perceived differently from ultrasound. Crickets were habituated to 5 pulses of 20 kHz at 10 dB for 100 ms. Trials were presented in blocks of 5, with a 500 ms inter-trial interval. The pulse train, presented at 20 kHz, was followed by a test pulse of 20 kHz as before. All pulses were presented to the left ear. The deceleration pulse was 100 ms, with a 50 ms rise time and a 40 ms fall time.

Pulses of any frequency habituated when presented from the direction opposite the habituating train, but only pulses below 17 kHz habituated when presented from the same direction as the habituating train. Thus, pulses below 17 kHz are habituated from 20 kHz, while those above 17 kHz are not distinguished from 20 kHz.

418.6 NEGATIVE THERMOKINESIS DURING FLIGHT IN THE LOCUST. R.M. Robertson* and C.T. Kuhnle, Dept. of Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

Locusts aggregate in regions with an optimal environmental temperature, yet there is currently no evidence for locomotor thermokinesis in locusts. We have investigated whether tethered flying locusts will steer relative to a radiant heat source.

Adult male Locusta migratoria were tethered and flown in front of a wind tunnel. Wing movements and postural adjustments of the abdomen and hindwings were monitored with high-speed cinematography (250 frames/s) and with video digitation (60 frames/s). In separate experiments, abdominal movements were monitored using a capacitative position transducer. Brooder lamps (250 watt) were placed on the left and right of the animal providing directional heat sources.

Within 1s of turning on a lamp, locusts made characteristic steering manoeuvres away from the direction of the source. Interposing a transparent acrylic barrier (1.2 cm thick) markedly reduced the steering responses. Neither covering the lights nor adding sand, nor interposing infra-red filters (Edmund Scientific, P60 033) between the locust and the lamp, had an effect on thermokinesis.

We conclude that flying locusts are attracted by heat sources by detecting infra-red radiation. We are currently investigating which structures are involved in mediating directional infra-red sensitivity.

418.7 SYNCHRONIES IN THE NORTH AMERICAN FIREFLY PHOTURIS CAROLINA. J. Cripps and A. H. Hurd, Department of Biology, Georgia Southern University, Statesboro, GA 30460 and Department of Physiology and Neurobiology, University of Connecticut Health Center, CT 06269-3028.

Photuris carolina shows discontinuous synchrony. Bursts of 5-8 flashes at 2 sec occur concurrently every 14-19 sec. When the terrain is flat and forested, the synchrony is always present. When the terrain is steep and forested, the synchrony is a wave synchrony. Thus, the same species of firefly is capable of showing unison or wave synchrony on the topographical conditions.

We studied unison synchrony by employing low level light videography and frame-by-frame playback analyses as well as photometric recordings. The latter was combined with computer acquisition and analysis in the field. Data obtained from field caged males revealed that group flashing began concurrently, ended concurrently, and the flashes within the burst occurred concurrently, resulting in the mechanisms responsible for this synchronic behavior, stimulated flashes were presented to individual fireflies at various times during their flash patterns. Stimulus flashes presented during the interburst interval enhanced or inhibited the next flash depending on when the stimulus was presented. Stimulus flashes presented during the interburst interval, however, had no noticeable effect.

We suspect that the interburst oscillator can be modulated through anticipatory (unison) or triggered (wave) mechanisms. We also suspect that the interburst oscillator, once released in the ethological sense, is not susceptible to feedback.

Supported by NSF grant IBN-9208709 and GRC grant 11939.


Periplaneta americana responds to abrupt touch of an antenna by turning away from the stimulus and running. This response occurs at very short latency, and the angle of initial turn is typically convex. We have noted that this response is not essential for escape turns (Comer et al., 1994; J Comp Physiol 174:17).

We believe that visual cues are actually used as touch of antennae. We were therefore interested in knowing if aspects of escape are influenced by vision.

We report here behavior studies on intact animals tethered in a high-speed locomotion tunnel, and electrophysiological studies of descending interneurons. When lateral eyes and ocelli were covered with opaque paint, animals turned reliably following antennal touch. But the duration of subsequent running was decreased. In another experiment, sighted animals were tested following response to a stimulus presented near an antenna. Animals responded by orienting an antenna toward the probe and touching it if the probe had high visual contrast with background. If the probe had low contrast, contact with the probe was at chance levels. These data indicate that cockroaches visually detect stimuli applied to the antennae, and that vision (while non-essential) may influence escape performance.

Intracellular recording and dye-injection revealed an interneuron, with soma in the brain, that responds to visual stimuli of the type used in behavior tests. In recordings from neck connective, the neuron typically responded by firing a train of spikes as a probe moved across the visual field of the contralateral eye. When antennal touch occurred, the firing of this neuron preceded that of descending sensory-motor interneurons. The cell's axon (15-20 μm in diameter) extends to the thoracic ganglia.

We suggest that while visual cues are not essential for touch-evoked escape, they may influence some elements of the escape response. The role of descending interneurons in such an influence is under investigation.

This research supported by NSF grant IBN-9221619.

418.9 TESTING FOR A POPULATION VECTOR CODE FOR WIND DIRECTION IN THE COCKROACH GIANT INTERNEURONS. R. Less, J. M. Cymb, Dept. Cell and Animal Biology, Hebrew University, Jerusalem, Israel.

One proposed mechanism by which a neural assembly codes direction stimulus is the vector population code (VPC). Originally observed in the monkey cortex, this is noted here in the cockroach escape system. Specifically, we test 3 bilateral pairs of giant interneurons (GI's) that respond directionally to wind stimuli and evoked as evasive turn. GI 3 responds most strongly to wind from the ipsilateral rear. Such wind makes large turns away. GI 3 to respond most strongly to wind from the ipsilateral rear. Such wind makes large turns away. GI 1 responses equally to both directions (Kolben and Cymb, Soc. Neurosci., Abst., 1993).

By a VPC, one represents a wind stimulus as a vector, whose direction is the cell's best excitatory direction, and whose length is the number of spikes evoked by the given wind stimulus. Vector summation of the GI's 1, 2 and 3 gives good agreement (± 20°) between the actual wind direction and that calculated using the VPC.

To help determine whether the cockroach actually uses a VPC, we modified the spars trans of individual GI's during wind-evoked turning behavior and determined the effects on the turn. Wind pulls from 90° right evoke, in a cockroach turned on a slick surface, turning movements to the left. We analyzed the initial leg movements and body's turning tendency using a high speed video (250 frames/s). During another puff from the same source, intracellular current pulses, to either right GI 3 or 2. Roughly doubling the number of spikes in GI 3 (from 6 to 12) has produced, to date, responses corresponding to larger turns to the left in 7 of 9 cockroaches, responses instead to GI 1 has produced spiral turns to the right, responses corresponding to smaller turns in 4 out of 5 cockroaches. These data are consistent with the predictions of a VPC, since increasing the number of spikes lengths a GI's vector and thus drives the direction of the wind specified by the VPC toward that GI's best excitatory direction.

418.10 SPONTANEOUS SELF-GENERATED COUNTERTURNS DURING TETHERED FLIGHT OF MALE MOTTIS, Manduca sexta. R. Baker*, M. Willis, E.A. Archer*. *Center for Neurogenic Communication Disorders and 1st. Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721

Flight behavior of mottis, Manduca sexta, is known to be modulated by odors and visual patterns. In plumes of female pheromone, for example, flight tracks of males assume a characteristic upward zigzagging pattern. Our laboratory is researching the sensori-motor basis of this pattern. We have recorded spontaneous activity patterns of tethered mottis in the 1st hour of their cirradian activity cycle, without specific olfactory or visual stimulation. Movement patterns were logged visually and videotaped. Synchronous EMG recordings from various wing muscules revealed unchanging movement patterns.

Mottis typically activated wing flapping (pre-flight warmup), followed by large amplitude wing beating indicative of tethered "flight," within the first 10 min. of scotophase. Flight continued in bouts from a few sec to 14 min. duration. During flight bouts, mottis mostly maintained their postural stability and were consistent with stable forward orientation. Often, however, they showed spontaneous changes in posture during wing beating, we interpret them as attempts to turn, including: (1) turning of the head and, (2) curling the abdomen in the direction of the intention, as well as (3) retraction of the wings on the inside of the turn, and protrusion of those outside the turn. Attempted turns were sometimes executed in rapid alternating succession, reminiscent of counterturning during unprovoked zigzag flying. These observations suggest a capability to organize counterturning intrinsically, rather than only in response to afferent inputs. Timing of movements and motor output patterns underlying these spontaneous turns are unique, and are parallel to those observed in males posturings. Further studies are necessary to determine the circuitry of these spontaneous movements, and to compare with similar patterns activated and modulated by visual and pheromonal stimuli. (Supported by NSF IBN 9216532).
418.11 MODULATION OF MOTOR PATTERNS IN A TETHERED MOTH DURING MULTI-MODAL STIMULATION. EE Wood* and EA Arbas. ARL Div. of Neurobiology, Univ. of Ariz., Tucson, Ariz. 85721

We conducted the flight behavior of the male sphinx moth, Manduca sexta, as a model for the generation and control of orientated locomotion. Of interest is control of pheromone-evoked "zig-zagging" flight of males searching for mates. This pattern of flight exhibits a consistent interturn interval as the male progresses upward. Previous results described changes in activity in the animal in response to free flying animals and reduced preparations from which EMGs of flight muscles could be monitored (Willis and Arbas, 1991; Katzaki and Arbas, 1990). This investigation describes aspect of the flight control in various multi-modal stimulations while recording electrical potentials in relation to specific pheromone neurons of particular motor nerves. Study of flight initiation shows that short bouts of stereotyped flight are evoked by short (< 1 s) exposures to flight. Short bouts of flight are also temporarily random and uncoordinated in central control of flight (flight after odor loss). These short bouts are useful for comparison to more complex flight patterns. Cholinergic neurons (Katzaki and Arbas, 1990) also produce stereotyped flight that are evoked by this type of flight. Visual stimuli simulating left and right turns can initiate flight with regular turning intervals. Visual stimuli combined with pheromone stimulation elicited frequency modulation of the flight rhythm reminiscent of that observed during free flight. We are continuing this study using more complex computer driven stimuli protocols accompanied by recordings of motor rhythms in the thoracic ganglia. (Supported by NIH NRSA F32 DC 00097-01 and NSF IBN 921562).

418.12 CAN NEUROGENESIS EXPLAIN MUSHROOM BODY REORGANIZATION IN THE BRAIN OF THE ADULT HONEY BEE? JL Strande, S.E. Fahrbach and G.E. Robinson*. Department of Entomology and Neuroscience Program, University of Illinois, Urbana, IL 61801. A reorganization of the mushroom bodies in the brain of the adult honey bee (Apis mellifera) is associated with behavioral development and colony labor (Wulff and Rehan, 1981; Nature 364, 238-240). To probe the proximate mechanisms of this neuroanatomical plasticity, we tested the hypothesis that neurogenesis occurs in the adult bee brain. Bees were exposed to the proliferation marker bromodeoxyuridine (BrdU) at 3 stages of behavioral development: one-day-old, nursing, and foraging. BrdU was administered in 3 ways: 1) into the brain corpus via injection into the abdomen, or in vitro, with brains incubated in bee culture medium containing BrdU. Immunohistochemical experiments completed to date revealed only one BrdU-labeled nucleus of a single mushroom body neuron in 2140 sections of tissue examined. Several controls were processed simultaneously with adult bee brain tissue. Nervous tissue from Manduca sexta larvae, in which neurogenesis is known to occur, revealed extensive BrdU labeling. Neurogenesis was also readily detected in nervous system tissue from honey bee larvae and pupae. These results indicate that neurogenesis occurs very infrequently or not at all in the adult bee brain. It does not appear that the previously observed mushroom body reorganization can be explained by neurogenesis. Supported by NSF grant IBN 9211366 and an NSF REU Award (SEF and GER) and NIMH grant MH42724-01 (GER).

419.1 MDMA INCREASES THE EXTRACELLULAR CONCENTRATION OF 2,3-DIHYDROXYBENZOIC ACID IN THE STRIatum: EVIDENCE FOR INCREASED HYDROXY RADICAL FORMATION. G.A. Gudelevy* and B.K. Yamamoto, Dept. of Psychiatry, Case Western Reserve University, Cleveland, OH 44106 It is well recognized that the administration of 3,4-methylenedioxymethylamphetamine (MDMA) results in a long-lasting depletion of brain 5-HT. It is suggested in the literature that the depletion of 5-HT is due, in part, to the acute and sustained release of dopamine (DA). The purpose of the present study was to examine whether the MDMA-induced depletion is in the extracellular concentration of DA in the striatum is accompanied by the formation of hydroxyl free radicals that result from DA autooxidation. Hydroxyl radical formation was assessed in dialysis samples from the striatum by quantifying the extracellular concentration of 2,3-dihydroxybenzoic acid following its conversion from salicylic acid. The dialysis probes were perfused with dialysis buffer containing 5 mM salicylate for 3 hrs at a volume flow of 500 nl/hr. The ip injection of MDMA (20 mg/kg) resulted in an immediate increase in the concentration of 2,3-DHBA which was 450% of that in vehicle-treated controls. An MDMA-induced increase in 2,3-DHBA formation was observed for at least 6 hrs. A 30 min application of MDMA (10 mg) by the dialysis probe also increased 2,3-DHBA concentrations which were similar in magnitude to those produced by systemic administration. In rats in which the MDMA-induced increase in DA release was attenuated by treatment with mazindol, the MDMA-induced increase in 2,3-DHBA formation also was significantly attenuated. These findings are consistent with the view that MDMA produces a DA-dependent increase in hydroxyl radical formation.

419.2 POTENTIATION OF MDMA-INDUCED DOPAMINE RELEASE AND SEROTONIN NEURON TOXICITY BY SEROTONIN AGONISTS. J.E. Nash, B.K. Yamamoto and G.A. Gudelevy. Deps. of Psychiatry and Neuroscience, Case Western Reserve Univ., Cleveland, OH 44106 The effect of serotonin (5-HT3) agonists, DOI and 5MeODMT (5-methoxy-N,N-dimethyltryptamine) on MDMA (3,4-methylenedioxymethylamphetamine)-induced release of dopamine (DA) in the striatum was studied in rats by the use of in vivo microdialysis. A concentric dialysis probe was inserted into the striatum and dialysis samples were collected every 30 min for 240 min. MDMA (2.6 mg/kg, sc) administration increased the release of DA in the striatum. The MDMA-induced increase in the extracellular concentration of DA was enhanced significantly in rats given either DOI (1 mg/kg, ip) or 5MeODMT (15 mg/kg, ip). Neither DOI nor 5MeODMT alone affected the extracellular concentration of DA in the striatum. The effect of DOI on the long term depletion of striatal 5-HT produced by MDMA also was investigated. The striatal concentration of 5-HT was reduced slightly, but not significantly, 7 days following the administration of MDMA (10 mg/kg, sc). However, 7 days following the concomitant treatment with DOI and MDMA the striatal concentration of 5-HT was significantly less than that in rats treated with MDMA alone or the vehicle-treated controls. It is concluded that activation of 5-HT3 receptors is an important determinant of the acute increase in extracellular DA and, consequently, the long-term depletion of brain 5-HT produced by high dose administration of MDMA.

419.3 THE STRIATAL NEUROTOXICITY INDUCED IN THE C57BL/6J MOUSE BY D-AMPHETAMINE IS BLOCKED BY RESTRAINT STRESS BUT NOT BY SUPPLEMENTAL CORTICOSTERONE. D.B. Miller* and J. P. O'Callaghan. U.S. EPA, RTP, NC 27711

Amphetamine (AMP) is a potent stimulant and restrained (ST) dopaminergic neurotoxocis. Stress affects AMP but its effects on AMP neurotoxicity are not well examined. Restraint stress activates the adrenal axis and elevates corticosterone (CORT) levels. Here, we determined if restraint or supplementation with CORT potentiated or attenuated the effects of d-AMP neurotoxicity. Astrogliosis, quantified by immunohassey of GFAP, was taken as a marker of neuronal damage. Body weight (BT) was also monitored as restraint can cause hypothermia and BT is a factor in AMP neurotoxicity. Female mice (6/group/case) were given SAL or 5-MeODMT at a dose of 20 mg/kg. For each BT, every 2 hrs beginning at 11:30 AM for a total of 4 inj. Mice were restrained in 50 ml centrifuge tubes, beginning at 0.5 hr prior to the 1st inj and until 1 hr following the last inj. BT of the water drinking rats was placed in the drinking water (20g/ml - 3.2 g/kg) 1 week prior to AMP inj and continued until the ST was obtained at 72 hrs post the last inj. BT was recorded prior to dosing and every 2 hrs following each inj. AMP caused hypothermia (1 - 2°C) and neural damage as indicated by a large (30%) increases in ST GFAs. Restraint totally blocked the d-AMP hypothermia as well as the GFAs increase. CORT had no effects. The data suggest an elevation in BT is important in d-AMP neurotoxicity and that neurotoxicity status may not play a role in AMP neurotoxicity. However, the dosage of CORT utilized was low and did not cause thymic invovlation. Higher dosages, as well as the elimination of circulating CORT, such as by adrenalectomy, should be investigated.

419.4 D-Amphetamine (AMPH) Levels in Caudate-putamen (CPU) Microdialysis After Doses That Produce Either Behavioral or Neurotoxic Effects. P. Clausing, B.R. Holson, W. Slikker Jr., B. Gough and J.F. Bowyer, NCTR/FDA, Jefferson, AR 72079-9502. Six mo. old Sprague-Dawley rats (4/group) were dosed with either 1 or 2.6 mg/kg AMPH sc to produce increases in locomotor activity and/or stereotypy. Forty to 60 min after 1 mg/kg, when motor activity was maximum, AMPH levels in the microdialysate were maximal at (mean ± SEM) 0.28 ± 0.04 μM. Forty to 90 min after 2.6 mg/kg, the microdialysate levels rose to a max. of 0.51 ± 0.09 μM. Stereotypic behavior started at this time and lasted for 1-2 hrs. In a second experiment, 6 and 12 mo. old rats (5/group) were given AMPH sc either 4.5 μg/kg in a 24°C environment (known to produce neurotoxicity) or 4.15 μg/kg in a 10°C environment (not toxic) at 2 hr intervals. It was hypothesized that in the cold environment the higher doses would produce higher CPU-extracellular AMPH levels and higher neurotoxicity levels. Peak levels were: 1.5 ± 0.3 μM (6 mo. old, 4.5 μg/kg, 24°C), 2.6 ± 0.7 μM (12 mo. old, 4.5 μg/kg, 24°C), 3.0 ± 0.4 μM (6 mo. old, 4.15 μg/kg, 10°C), 1.1 ± 0.5 μM (12 mo. old, 4.15 μg/kg, 10°C). Thus, 12 mo. old rats had significantly higher AMPH levels than 6 mo. old. Cold room rats dosed with triple the dose had 1.4-2.0 times the AMPH levels seen in the 24°C rats. Thus the efficiency of the microdialysis probes was estimated to be 15-20%. Thus, approximately 2 μM extracellular AMPH is necessary to produce behavioral effects. The data are preliminary and the results are similar to those in the CPU while levels after neurotoxic doses are between 10 and 30 μM.
419.5

Using Orthophthalaldehyde (OPA) and 3-Mercaptopropionic Acid (MERA) Derivatization to Quantify D-Amphetamine (AMPH) Levels in Brain Tissue by High Performance Liquid Chromatography (HPLC) J.F. Brower and P. Clauzing, NCTR/FA, Jefferson, AR 72079-9502.

Rats implanted for microdialysis in the striatum were dosed with AMPH (1 to 15 mg/kg, sc). The microdialysate buffer consisted of 140 mM NaCl, 1.5 mM K2HPO4, 1.5 mM KC1, 1.5 mM MgCl2, 1.25 mM CaCl2 and 10 mM glucose, pH 7.4. Dialysate was collected continuously at 20 min intervals (10 ml/hr) for 4 hrs starting at 2 hrs, prior to dosing. The samples were either stored at -70°C or analyzed immediately by HPLC. AMPH levels were determined by derivatizing 10 to 20 μl microdialysate with 20 μl of 0.1 M borate buffer, pH 9.4, containing 5 mg OPA and 40 μL MERA per ml and 70 to 90 μl H2O. Then 75 μl of the derivatized sample was separated on a C18 column using 50 mM potassium phosphate buffer, pH 5.5, and a gradient of 35% to 65% methanol with 1.5 ml/min flow rate. Fluorescent detection (340 nm λ excitation, 440 nm λ emission) was used. The AMPH derivative had a retention time of 14.4 min which was 2 min more than all the amino acid derivatives and there were no peaks within ±1.5 min of the AMPH derivative. Very little p-hydroxyamphetamine was detected in the microdialysate after AMPH sc. Microdialysate buffer spiked with AMPH to obtain 0.1 to 10 μM AMPH concentrations was used to quantify microdialysate AMPH levels. The detection limit was 0.05 μM AMPH in 20 μl of microdialysate, equivalent to 135 picograms. From 0.27 to over 27 nanograms AMPH can be quantified in microdialysate using these methods.

419.7

PRENATAL METHAMPHETAMINE CAUSES SPECIFIC METABOLIC CHANGES IN THE BASAL GANGLIA SEEN AT MATURATION. A.D. Heenanart and S. Caldecott-Hazard, Department of Pharmacology, Seton Hall University, School of Graduate Medical Education, South Orange, NJ 07079

Methamphetamine exposure in utero has been shown to produce profound alterations in the adult brain monochrome system. These effects are also reflected in subtle changes in specific adult tasks. Human maternal methamphetamine abuse may have a similar impact on the fetal brain that may persist into adulthood. We sought to model the drug's developmental effects chronically on fetal rats by i.p. The "C" deoxyglucose technique (DG) was used to measure adult brain metabolism and as a sensitive indicator of long term changes in neuronal function. In utero methamphetamine (10 mg/kg) was administered throughout gestation. Drug related maternal anorexia, malnutrition, altered blood pressure/ temperature and postnatal care were controlled by using rats made tolerant to the drug's side effects and by cross fostering. At birth, the drug exposure ended and the brain metabolism of the drug-free adult offspring were behaviorally assessed and mapped with DG. An increased rate of glucose utilization was seen in the globus pallidus of the perinatally treated animals. No changes were seen in other areas of the basal ganglia, lateral habenula or medial dorsal prefrontal cortex. These results are in contrast to our previous observations in these areas following amphetamine withdrawal in adults. However, both paradigms produced a similar decrease in open field activity.

In utero drug treatment produces specific alterations in brain metabolism in the adult offspring at 30 days of age. These changes were accompanied by both neurological and performance deficits and to the point of the production of an altered adult neurochemical and behavioral state that can be attributed to the prenatal neurotoxic effects of methamphetamine on specific brain sites.

419.8


Treatments with high doses of methamphetamine (METH) leads to well-described long-term decreases in serotonin and dopamine in certain regions of the brain. Despite quite dramatic reductions in these neurotransmitters, very few baseline behavioral changes have been observed in rats following neurotoxic regimens of METH. The present study was undertaken to examine behavioral and neurochemical consequences of METH neurotoxicity.

Adult, male Sprague-Dawley rats received injections of saline or METH (6.5 mg/kg sc) four times daily, at two hour intervals, for ten days following treatment behavior was assessed in a large (4 x 4 x 4 ft) plexiglass open field apparatus. Animals were placed in the center of the open field and behavior was observed for ten minutes. Four days following behavioral testing, animals were sacrificed, and the brains examined by immunocytochemistry, receptor autoradiography and in situ hybridization for changes in serotoninergic function.

As has previously described, METH produced small, but significant decreases in serotonergic indices in several brain regions, including septocs, hippocampus and caudate-putamen. Other areas, such as substantia nigra appeared to be spared. Animals that had received METH showed a significant increases in locomotor activity and talking, relative to saline controls. The increases in behavior were seen primarily toward the end of the ten minute session, and occurred despite the fact that animals had been drug-free for ten days. The results suggest that METH neurotoxicity produces significant changes in locomotion and exploration. These changes may be due to decreases in serotonergic and/or dopaminergic function. (Supported by NIDA DA02265 and NIMH MH2251. KAT and DTC are recipients of NARSAD Young Investigator Awards).

419.9


In experimental animals, methamphetamine (METH) causes pronounced neurotoxic effects to monoaminergic neurons. Studies have demonstrated that various pharmacological agents prevent METH-induced neuroapathy indicating that several processes may be involved in mediating the toxicity of METH. Several neurotoxic effects of METH such as brain-derived neurotrophic factor (BDNF), have significant roles in development, maintenance and survival of dopaminergic neurons. Roles for neurotrophic factors in supporting the survival of dopaminergic neurons in the basal ganglia remain to be determined, especially under conditions of stress or injury. Therefore, our aim was to characterize the dynamics of BDNF expression during and after an insult to nigrostriatal dopaminergic neurons by METH in mice. Our results indicate that expression levels of BDNF increase approximately 2-4 fold in the mesencephalon and cortex 24 hr after initiation of a neurotoxic regimen of METH. These results demonstrate that stress or injury to dopaminergic neurons in the basal ganglia is a regional expression of neurotrophic factors (i.e. BDNF) in an adult mouse brain.

419.10

METHAMPHETAMINE SENSITIZATION INCREASES VULNERABILITY OF THE PREFRONTAL CORTEX TO METHAMPHETAMINE TOXICITY B.N. Yamamoto* and S.E. Shephard, Deps. of Psychiatry and Neuroscience, Case Western Reserve Univ., Cleveland, OH 44106.

Sensitization to the behavioral and neurochemical effects of stimulants occurs following a low dose intermittent pretreatment regimen. This is manifested as an increase in behavioral stereotypy and mesocorticolimbic dopamine (DA) function subsequent to the administration of an acute low dose challenge. These effects occur without evidence of dopamine (DA) depletion. The purpose of this study was to examine the sensitization regimen of methamphetamine (METH) enhances DA release and the subsequent depletion following neurotoxic doses of METH. Male rats were injected once daily for 7 days with METH (2mg/kg, i.p.) or saline. Following a 7 day withdrawal, the rats were challenged with 3 subcutaneous injections of METH (7.5mg/kg), each injection given 2 hours apart. Microdialysis was used to measure extracellular levels of dopamine (DA) and cortical serotonin (5-HT) in the pfc (pc) of awake rats. Rats were sacrificed 4 days later and DA tissue content was assayed. In METH pretreated rats, DA release in pfc but not in snt was significantly increased during the METH challenge as compared to pretreated with saline (p<0.02). One week later, this group of rats also exhibited a greater depletion of DA in pfc (p<0.02) compared to the saline pretreated group. Saline and METH pretreated rats given a saline challenge were not different from controls. Conversely, striatal DA was significantly less depleted by a METH challenge in METH pretreated compared to saline pretreated rats (p<0.02). These data provide evidence that pfc DA neurons are preferentially susceptible to the neurotoxic effects of METH in rats previously sensitized to METH. Augmented DA release in the pfc may be partially responsible for the dopamine depletion following a METH challenge.

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419.11

**DRUGS OF ABUSE: CNS STIMULANTS—TOXICITY**

**DOPAMINE AND GLUTAMATE EFFLUX ARE INCREASED IN PREFRONTAL CORTEX OF RATS SENSITIZED TO METHAMPHETAMINE**

S. E. Stephen{ }and B. K. Yamamoto. Dept. of Psychiatry and Neurosurgery, Case Western Reserve Univ., Cleveland, OH 44106.

Behavioral and neurochemical sensitization occurs following intermittent, low doses of psychostimulants such as methamphetamine (METH). This is manifested as behavioral stereotype and increased mesocortical dopaminergic function following administration of a challenge dose. No direct comparisons have been made with regard to whether the increased dopaminergic (DA) response in vivo is mediated by an impulse-dependent or independent mechanism. Furthermore, it is not known if release of other neurotransmitters is affected by this pretreatment regimen of METH. The purpose of this study was to investigate if DA and glutamate (glu) release in the prefrontal cortex (pfc) is augmented in METH sensitized rats upon a low challenge dose of METH or potassium (K+)-stimulated. Male rats were pretreated once a day for 5 days with METH (2mg/kg, i.p.) or saline. Following a 7 day washout period the rats were challenged with either a low dose METH injection (2mg/kg, i.p.) or K+ (80mM) infused into pfc. Microdialysis was performed in the diencephalon (DA) and pfc. DA infusion into the pfc of METH pretreated rats increased extracellular DA and glu levels significantly from baseline values (p<0.02). These increases were greater than in saline pretreated rats (p<0.05). The low dose METH challenge significantly increased extracellular DA but not glu concentrations to a greater extent in METH pretreated compared to saline-pretreated rats (p<0.02). These data provide evidence that a low dose pretreatment regimen of METH enhances DA transmission in the pfc through both impulse- and drug-dependent processes. In addition, enhanced glu release in pfc may play an important role in the behavioral effects associated with METH sensitization.

419.12

**INFLUENCE OF PRIOR BEHAVIORAL SENSITIZATION ON THE NEUROTOXIC EFFECTS OF METHAMPHETAMINE**

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Sensitization to psychomotor stimulant drugs is associated with alterations in brain dopamine (DA) neurotransmission. DA release has been implicated in the neurotoxic action of methamphetamine (METH). The purpose of the present study was to determine whether prior sensitization influenced the neurotoxic effects of METH on brain DA and serotonin (5-HT) neurons. To produce sensitization, mice were treated with METH (15 mg/kg, i.p.) once daily for 15 consecutive days. Control animals were treated identically with saline. One week later, the development of behavioral sensitization was documented by measuring a leftward shift in the dose-response curve for stereotype in METH-treated mice. After an additional one-week drug-free period, sensitized animals, along with controls, were treated with neurotoxic doses of METH. Seven days later, the animals were sacrificed and indexes of dopaminergic and serotonergic neuronal integrity were measured. Sensitization did not augment either the dopaminergic or serotonergic neurotoxic effects of METH in the striatum. To the contrary, the sensitizing regimen of METH appeared to render the animals partially tolerant to the drug's DA neurotoxic effects.

These results indicate that a chronic regimen of METH that induces behavioral sensitization does not render brain dopamine and serotonin neurons more vulnerable to METH's neurotoxic actions. Further, the partial tolerance observed suggests that the neurochemical mechanisms underlying the expression of behavioral sensitization may at least partially overlap those mediating METH's neurotoxic actions.

419.13

**EFFECTS OF d-FENFLURAMINE AND m-CHLOROPHENYLPIPERAZINE ON ACUTE 5-HT RELEASE AND LONG-TERM 5-HT DEPLETION IN RAT BRAIN**

M. H. Baumann, M. A. Ayestas, R. B. Rothman. Clinical Psychopharmacology Section, IRP, NIDA, NIH, Baltimore, MD 21224.

The serotonin (5-HT) agonists d-fenfluramine (FEN) and m-chlorophenylpiperazine (mCPP) are reported to release neuronal 5-HT by a Ca"++-independent, fluxeine-reversible mechanism. In the present work, we compared the acute effects of FEN and mCPP on extracellular 5-HT and dopamine (DA) using in vivo microdialysis methods in rats. The long-term consequences of repeated injections of FEN or mCPP on 5-HT neurotransmission were also examined. In the acute study, FEN or mCPP (10, 100 μM) was infused into the nucleus accumbens of conscious rats via microdialysis probes and monoamines were assayed in dialysate samples by microbore HPLC-EC. FEN and mCPP caused equivalent, dose-dependent increases in 5-HT, but not DA, at 1 and 10 μM doses. At the 100 μM dose, both drugs elevated dialysate DA as well as 5-HT. In the long-term study, rats were treated with FEN or mCPP (0, 3, 10 mg/kg, i.p.), one injection every 2 hr for 8 hr. Two weeks after the repeated dosing regimen, FEN-treated rats exhibited marked depletions in tissue levels of 5-HT (78%) and 5-HIAA (72%) in frontal cortex, whereas mCPP-treated rats showed no signs of 5-HT depletion. Thus, mCPP appears to enhance acute 5-HT release, in a manner similar to FEN, without causing damage to 5-HT nerve terminals. The present data suggest the possibility that 5-HT release per se may not be the causative factor in the serotonergic neurotoxicity associated with FEN and other substituted amphetamine derivatives.

419.14

**BRAHMI: AN ANCIENT BUT MODERN HERB**

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Brahmi (Centella asiatica) is a well recognized herb with a range of traditional uses in Indian and other Ayurvedic systems of medicine. Recently, several of these uses have been validated by scientific research. For example, Brahmii has been shown to improve cognitive performance in animal models of Alzheimer's disease and depression. Furthermore, Brahmii has been shown to have anti-inflammatory and immune-modulating properties, which may contribute to its beneficial effects on brain function.

419.15

**BEHAVIORAL AND NEUROTOXIC EFFECTS OF METHCATHINONE**


Cathinone is a naturally-occurring amphetamine analog found in the Khat plant. Like amphetamine, cathinone has been abused and, in high doses, is selectively toxic to brain dopamine (DA) neurons. Methcathinone ("Cat") is a synthetic cathinone derivative that has recently surfaced in the illicit drug market. Because it contains an asymmetric carbomethoxyl group, methcathinone is available in D and L forms, and it is the L form that is used recreationally. The present studies evaluated the pharmacologic and neurotoxic properties of methcathinone stereoisomers in laboratory animals. Pharmacologic studies in mice indicated that L-methcathinone was approximately five times more potent as a locomotor stimulant than D-methcathinone. By contrast, in neurotoxicity studies, the D isomer proved more toxic to DA neurons in mice. To evaluate methcathinone's neurotoxicity in vivo, we performed a series of experiments. The results indicated that methcathinone was toxic to DA neurons in a dose-dependent manner. The neurotoxic effects of methcathinone were dose-dependent, with the L-isomer being more toxic than the D-isomer. These results indicate that methcathinone is a potent neurotoxin, and that the L-isomer is the more toxic form. In conclusion, our findings suggest that methcathinone is a promising target for the development of new amphetamine derivatives with therapeutic potential.
MEDIOL SEPTAL SECTIONS ENHANCE LOCOMOTOR SENSITIZATION TO AMPHETAMINE. J. E. Kelsey* and J. A. Grabiec. Dept. Psychology, Bates College, Lewiston, ME 04240.

Sensitization to the locomotor-enhancing effects of several drugs appears to critically involve the dopaminergic mesolimbic pathway from the VTA to the n. accumbens. Such locomotor sensitization appears to be modeled by projections from the hippocampus to the n. accumbens. In this experiment, we examined sensitization to the locomotor-enhancing effects of amphetamine in rats with lesions of differentially selected brain regions. Each subject received a single intraperitoneal injection of amphetamine and saline to five rats. During acquisition, the rats were injected i.p. with 1.0 mg/kg amphetamine sulfate or isotonic saline and immediately placed in a 58 cm black open field where distance travelled was measured during 2 hr sessions spaced 2 days apart. All rats were then injected i.p. with 0.4 mg/kg amphetamine and isotonic saline 2 days apart. Although there were no differences between the sham-operated control and lesioned rats in the acute locomotor response to either 0.4 or 1.0 mg/kg amphetamine, enhanced locomotor sensitization and conditioned sensitization were observed in the rats with medial septal lesions during acquisition and testing. These data suggest that the input from the septum to the hippocampus normally serves to inhibit sensitization to the locomotor-enhancing effects of amphetamine. Perhaps this projection also acts to inhibit sensitization to the rewarding effects of amphetamine.

Effect of chronic amphetamine on the behavioral dose-response curve. P.K. Randall*, P. Hodge, J.S. Randall College of Pharmacy and Inst. for Neuroscience, Univ of Texas, Austin, Texas 78712.

The response to amphetamine (AMPH) increased with repeated administration. With drugs such as AMPH producing curvilinear dose-response curves and multiphasic time courses, sensitization-induced changes may be difficult to interpret.

The purpose of this study was to determine whether sensitization could be more simply modeled by a shift in the dose response curve rather than as an alteration in the magnitude of response. Sprague-Dawley rats were injected in the home cage with 5 mg/kg AMPH (IP) every 4 days for a total of 5 treatments. For the last treatment the response to 0, 0.5, 1, 2, 4, and 8 mg/kg AMPH was determined. Locomotion and rearing were assessed using computer and stereotaxic ratings assigned every six min for two hours prior to, and four hours following injection.

Locomotion and rearing showed the typical response patterns to each treatment level and the response curve was shifted to the left in the overall dose-response curve. This shift systematically decreased with time until at 60-90 min there were no differences between the groups. The reciprocal of the ED50s in both groups fell exponentially following 1 hr. The sensitized animals were marked by a more rapid decrease in this measure than the control animals.

DRUGS OF ABUSE: CNS STIMULANTS—BEHAVIOR.

340.3

GENETIC ANALYSIS OF METHAMPHETAMINE'S EFFECT ON FEEDING BEHAVIOR IN BXD RECOMBINANT INBRED MICE. S. Angel-Gade* and J.K. Belknap. Dept. of Medical Psychology, Oregon Health Sciences University, Portland, OR 97223.

Amphetamines at low doses have been shown to produce anorexia in both humans and animals. To identify the genes mediating this effect, BXD recombinant inbred mice were tested on a five day food restriction paradigm which resulted in stable food intake. Mice from each strain were then divided into two equal groups. On Day 6, Group 1 was given 4.0 mg/kg methamphetamine (MA), str, whereas Group 2 was given the saline vehicle. On the next day (Day 7), both groups were given MA. Consumption was measured for the next 240 minutes. Significant drug and strain effects for the 24 strains tested were seen. Food consumption was decreased on average by 54%. In the extreme strains, food consumption was decreased by 90% (Fv-2), 11 (D1/Mi2), 12 (D12Nw1), 13 (Tpm), 15 (D13M6), 18 (D18M15) and 19 (D19Bv1). Verification of these loci using PCR in an independent F2 population is ongoing. Supported by NIDA DA07262 and Contract DA277-90-MJ-4565.
GENETIC RECOMBINATION OF AMPHETAMINE-INDUCED MOTOR BEHAVIORS. A. Fleischner, C. Vedantam* Laboratory of Neurobehavioural Genetics, National Institute of Mental Health, Bethesda, MD 20892 and *New York University Medical Center, NY 10016; USA

Quantitative Trait Loci (QTLs) responsible for high and low expression of murine mesencephalic tyrosine hydroxylase activity (TH/MES) were transferred onto C57BL/6JByJ (B6) inbred mouse strain background by successive backcrosses with concomitant selection for the extreme expressions of TH/MES (Vedantam, 1990). Subsequently, Artificially Selected Congenic Recombinant Inbred (ASCR) strains were derived from the selection lines. To investigate the effects of the gene transfer, locomotor activity (distance covered) and (D) and frequency of occurrence of rearing on the hind legs (F) were recorded for 30 min after i.p. injections of 0, 2.0, and 4.0 mg/kg D-amphetamine in the progenitor strains (B6 and BALB/cJ) and in two B6.C animal model lines (N=10). Analysis of the data indicates that the D/R ratio was significantly higher in the BALB/cJ strain than in the B6 strain. Similar D/R values were observed for the B6 and the B6.C lines at 0 and 2.0 mg/kg D-amphetamine. After administration of 4.0 mg/kg D-amphetamine the four strains fell into two classes, each class consisting of a progenitor and a B6.C line. The results are consistent with the hypothesis that both TH/MES and amphetamine-induced behaviors are affected by the same QTLs.

D-AMPHETAMINE (AMPH) DECREASES SYMPATHETIC NERVE DISCHARGE (SND) IN ANESTHETIZED RATS. W. Liu, M.C. Custaway and K.J. Virenker* Dept. of Pharmacology and Experimental Therapeutics & Alcohol and Drug Abuse Center, LSU Medical Center, New Orleans, LA 70112.

Studies in this and other laboratories have shown that cocaine, a well know sympathomimetic, decreases rather than increases SND in anesthetized and conscious animals. The purpose of this study was to determine whether AMPH, another sympathomimetic, would also decrease SND in pentobarbital-anesthetized rats. The studies were performed in male Sprague-Dawley rats (275-325 g). Cannulae were placed in the femoral artery and vein. The trachea was cannulated and the rats mechanically ventilated. Splanchnic SND was recorded using bipolar platinum electrodes (0.303-KHz bandwidth). Two groups of rats (n=5 each) were used. One group received 0.01 and 0.5 mg/kg AMPH, and the other 0.1 mg/kg AMPH intravenously. AMPH dose-dependently decreased SND (-4.2 ± 85.5%). The duration of the SND responses ranged from 1.4 ± 0.6 to 6.8 ± 0.5 min. AMPH also dose-dependently increased heart rate (39 ± 0.6 bpm, 1.4 ± 0.6 to 18 ± 7.2 min duration). Doses of 0.01 and 0.1 mg/kg AMPH increased mean arterial pressure (MAP), 18 ± 2 mmHg max.). Doses of 0.5 mg/kg AMPH increased (32 ± 3 mmHg, 3 ± 0.5 min duration) and then decreased (-36 ± 7 mmHg, 48.5 ± 11 min duration) MAP. These data show that AMPH, like cocaine, decreases SND in anesthetized rats. These findings suggest that an increase in sympathetic outflow is not responsible for the HR and pressor responses elicited by AMPH. (Supported by NIDA DA08255).


The effects of injections of either d-amphetamine or quinpirole, the D2 dopamine receptor selective agonist, on the development of a lever-touch response in an autoshaping paradigm were investigated. Male Sprague-Dawley rats received daily, post-session, intraperitoneally administered injections after 10 pairings of the presentation of a retractable lever (6 cm) and the delivery of a food pellet (USB). Reinforcement delivery was immediate if the subject contacted the lever, otherwise, the pellet was delivered upon lever retraction. Amphetamine, at a dose of 3 mg/kg (E=1.35=65.5, p < 0.0001) impaired the development of the lever-touch response, as compared to saline treated control rat, and quinpirole at a dose of 3 mg/kg [F (1.43)= 30.7, p <0.001] completely abolished the acquisition of the response. Neither drug affected acquisition at a lower dose (0.03 mg/kg). Administration of the higher doses of amphetamine (1 mg/kg) and quinpirole (2 mg/kg) also impaired responding, although the impairment lability was due to dose effects of the drugs. Initiation of either quinpirole or amphetamine treatment after complete acquisition of the autoshaping task did not affect responding. Taken together these results indicate that post-session administration of amphetamine impairs and quinpirole blocks the acquisition of an autoshaped lever-touch task, and that the impairment is not due to an aversive action of the drug on performance (Supported by DA 06192 to JLM and MI 15860 to EAP).

NEUROPHARMACOLOGY OF AMPHETAMINE AND ANTIPSYCHOTIC DRUGS IN NUCLEUS ACCUMBENS AND AMYGDALA OF SOCIALLY INTERACTING RATS: Z. B. Wang, M. Bonta, and G. V. Bebec. Program in Neural Science and Dept. of Psychology, Indiana University, Bloomington, IN 47405.

A neuroethological approach has been used to compare the effects of haloperidol and clozapine on motor, social, and motivational behavior of amphetamine-treated rats. Adult, male rats were tested simultaneously in groups of three -- were exposed to a relatively enriched environment. In each tested group, one rat received amphetamine in nucleus accumbens (NAC) or amygdala (AMG) followed within 15-20 min by either haloperidol or clozapine. The other two rats were used as companions (no infusion) and controls (saline). Behavior patterns were classified into three main elements: stereotyped motor behavior, social contacts, and motivational behavior. Each element consisted of several behavioral subcategories. Behavior was recorded on video tape and rated by a trained observer. The results indicate: 1) amphetamine induces more stereotyped motor behavior in NAC rats than in AMG rats; 2) clozapine effectively reduced all amphetamine-activated stereotyped motor, social and motivational behavior in both NAC and AMG rats, while haloperidol was less effective in the same way; and 3) the effects of both amphetamine and the antipsychotic drugs showed marked individual differences. A neuroethological analysis can provide important new information on the mechanisms underlying the complex behavioral effects of these and other drugs known to alter dopamine transmission. Supported by USPHS Grant, DA 02451.


We investigated whether self-administration of amphetamine could be supported by local administration of the drug into the n. accumbens via a microdialysis probe, and whether amphetamine self-administration alters adaptations in gene expression. Male Sprague-Dawley rats previously autoshaped to respond on a lever for food were implanted with cannulae in the n. accumbens. After surgical recovery, subjects received 60 lever presentations on a 5 min fixed-interval schedule. Lever responses were reinforced by 1 min intra-accumbens infusions of either saline or Riniger's solution. Lever-touch responding was maintained by delivery of 12 μg/g amphetamine, but not Riniger's solution, into the right n. accumbens via a microdialysis probe. Videotape analysis indicated that rats reinforced with amphetamine were more active than rats reinforced with Riniger's solution. Drug-induced changes in gene expression were examined by isolating mRNAs from either the drug-infused lever-touch responses or from the contralateral control lever. This subtraction yielded numerous responsive mRNAs that are being characterized. These results suggest that amphetamine self-administered via microdialysis probes into the n. accumbens maintains conditioned lever-touch responding, and that a single 5-hr self-administration session may induce new gene expression. (Supported by DA06192, DA04195).

DAILY PRETRAINING ADMINISTRATION OF D-AMPHETAMINE IMPAIRS ACQUISITION OF A CLASSICALLY CONDITIONED LEVER-TOUCH RESPONSE IN RATS. R.R. Rule, P.J. Janak and J.L. Martinez, Jr. Department of Psychology, University of California, Berkeley, CA 94720.

We previously found that pretraining administration of amphetamine does not affect performance of a classically conditioned lever-touch response. We now report that pretraining administration of d-amphetamine impairations acquisition of this same response. Sprague-Dawley rats were classically conditioned to touch a retractable lever (2 cm). This immediately provides the delivery of a reward pellet. A retractable lever was presented to subjects for ten seconds. Upon retraction of this lever, rats received a pellet of food. There was no contingency between food presentation and lever-touch responding. This procedure was repeated ten times daily with an intertrial interval of 45 sec. Animals were run for ten consecutive days. Rats received a daily i.p. injection 5 minutes prior to each training session. Animals receiving 3 mg/kg amphetamine performed significantly fewer lever-touches than animals receiving pretrial injections of saline. The performance of the amphetamine animals increased from an average of 1.4 lever touches on Day 1 to 2.8 lever-touches on Day 10. This increase is less than that of saline-treated animals who increased from an average score of 0.9 on Day 1 to 5.9 on Day 10. The acquisition rate in the control rats is less than that seen in instrumental conditioning or combined classical and instrumental conditioning (autoshaping). This research is supported by a Ford postdoctoral fellowship (RRJUnd DA06192) (JLM).
240.13

Methamphetamine (MA) administered to rats in high doses causes neurotoxic injury to dopaminergic and serotonergic neurons (De Vito & Wagner, 1986; Bicknell & Wightman, 1989). Previously we reported that MA in operant discrimination reversal learning (Cooper et al., 1991), and in delayed alternation T-maze performance (Cooper et al., 1992, 1993). In the current study, we examined in a delayed non-match to position task using a novel, computerized, touch-screen equipped operant conditioning apparatus (Marlatt, Butt & Dougherty, 1987). Rats received a total of four subcutaneous injections of either DA (1.5 mg/kg) or saline, with 2.5 h intervals between injections. Upon recovery, animals were reduced to 85% of their free-feeding weight and trained to activate a computer touch-screen for food reinforcement. An automated version of the T-maze alternation paradigm (see Cooper et al., 1992) was then implemented in two phases. In the first phase, animals were reinforced for pressing a rectangular stimulus located on either the left or right side (sample position) of a touch-screen equipped monitor. Following a delay of 1 s, a pair of rectangular stimuli appeared on the monitor and animals were reinforced only for selecting the stimulus located on the opposite side relative to the sample. Upon reaching a criterion of 85% correct, animals began the second phase of training where delays of 1, 10, 20, 40, and 80 s intervened between the presentation of the sample and choice stimuli. Compared to controls, acquisition performance in MA-treated animals was impaired at delay intervals of 1, 10, and 20 s (MANOVA; p < .05). Results suggest that high doses of MA cause lasting impairments in reinforced alternation performance and that these impairments are exacerbated by temporal delays. (GSH supported by NIMH R43-53-33).

240.15
EFFECT OF REPEATED METHAMPHETAMINE PRETREATMENT ON FREEZING BEHAVIOR INDUCED BY CONDITIONED FEAR STRESS. T.Tsuchiya. Dept. of Psychiatry, Hokkaido Univ. Sch. of Med., Sapporo 060, Japan.

We examined the effect of methamphetamine pretreatment on conditioned fear in mice Winter-Kremer. Rats received methamphetamine or the vehicle according to the repeated escalating dose schedule (1.25, 2.5, 3.75, 5 mg/kg s.c. x/2 every other day for a week). After the drug treatment period, the rats were exposed to electric foot-shock (2.5 mA for 30 min) for two days. Twenty-four hours after the last foot-shock session, the rats were again placed in the shock box without shocks for 5 min. Methamphetamine pretreatment significantly increased conditioned freezing behavior, suggesting that rats previously exposed to chronic methamphetamine are more sensitive to subsequent psychological stress than control rats. Repeated methamphetamine treatment did not decrease basal anxiety and serotonin concentrations in the brain. Furthermore, co-administration of MK-801 (non-competitive NMDA antagonist), amphetamine acid (amphetamine upake inhibitor) or flunitrazepam (serotonergic uptake blocker) did not alter the enhanced freezing behavior. Taken together, it seems that methamphetamine-induced hypersensitivity to anxiety is not due to the toxic effect of methamphetamine. While co-administration of SCH23390 (D1/D5 receptor) or raclopride (D2/D3 receptor) had no effect on the methamphetamine-induced increase in freezing, co-administration of YM-09151-2 (D2/D3 antagonist) prevented this increase. These results suggest that methamphetamine-induced anxiety levels is mediated by D2-like receptors.

240.17

The stereoisomers of the designer drug 3,4-methylenedioxymethamphetamine (MDA) have been reported to have distinct stimulus properties: (+) MDA is presumably similar in its effects to amphetamine while (-) MDA is more closely related to hallucinogens, such as DOM. While MDA is structurally similar to amphetamine, its behavioral effects appear to be mediated by both dopamine and serotonin. We have recently developed evidence which suggests serotonergic dependent neurotoxic effects of MDA may enhance the fenfluramine-like effects of a related compound, MDMA.

In the present study, we examined the effects of d-fenfluramine administration (6.0 mg/kg b.i.d. for four days) on the discriminative stimulus properties of the stereoisomers of MDA. Male Oregon-eggshell rats were trained to discriminate either (+) MDA (1.5 mg/kg/12 h) or (-) MDA (1.5 mg/kg/24 h) from saline in a two-lever operant task. Two groups of animals (20/group) were treated daily with 25 -110 mg/kg d-Fenfluramine and the training drugs (0.19-0.75 mg/kg) were administered during independent test sessions prior to administration of the d-fenfluramine regime.

In contrast to previous suggestions that (+) MDA may activate to amphetamine, the present results indicated that d-ambitamine did not substitute for either isomer of MDA. In addition, while (+) MDA substituted completely for (-) MDA, no evidence was found for the substitution of d-Fenfluramine for (+) MDA. These results suggest that d-ambitamine induced neurotoxicity may influence the cue properties of the stereoisomers of MDA, making these more similar. Although there were noted increases in drug appropriate responding during amphetamine pretreatment, such increases could not be attributed to the effects of d-fenfluramine treatment.

240.18

Effects of environmental inhibition of the methamphetamine (MA)-induced behavior of MA-treated rats were investigated. MA-induced sensitization of expression of behavioral sensitization were examined. Rats received daily injection of MA for 10 days (1 mg/kg, sc). Half of them were immediately returned to the home cage (the group) and the other half were individually confined in a cylinder with a diameter of 13 cm (narrow cage group) for 3 hrs after each injection. The third group received saline in the home cage. The rats were sacrificed on the 17th day or 17-18 day withdrawal period, and were readministered with MA (1 mg/kg or 0.5 mg/kg). The normal cage group showed significant enhancement in the motor activity. However, they showed significantly intensified stereotyped behavior compared with the saline group. Microdialysis studies revealed no enhancement in the ability of MA to increase dopamine release from the nucleus accumbens in the normal as well as narrow cage group compared with the saline group, when examined after either a 7-8 day or 17-18 day withdrawal period. These results suggest that environment inhibition of actual movement under the drug effect modifies the pattern and character of behavioral sensitization. The biochemical basis of the differential behavioral sensitization and the environmental modification of its expression remains unknown.

240.20
EVIDENCE THAT HIGH AND LOW RESPONDERS TO NOVELTY SHOW DIFFERENCES IN BEHAVIORAL RESPONSES TO DEXAMPHETAMINE AND ETHANOL. M.A. Caron* and A.R. Coles. Ohio State University College of Medicine, Columbus, OH 43210.

The amnestic make-up of the ventral and dorsal striatum differs between high (HR) and low (LR) responders to novelty (Caron et al., 1990). These two types of individuals normally co-occur in unselected outbred populations of Winter rats. These rats are known to react differentially to self-administration of dexamphetamine; they also show distinct locomotor responses to dexamphetamine (Science 245, 1511, 1989). The present study had two purposes. First, it was investigated whether dexamphetamine-induced stereotyped behavior also differs between these rats; for the sake of comparison, a study of the locomotor effects of dexamphetamine was included. Second, we investigated to what extent these rates differently react to ethanol, since this drug is also known to influence the amnestic activity in the striatum. Male HR and LR rats were selected as an open field paradigm (Brain Res. Bull. 24, 49, 1990). Stereotyped tracking behavior (STB; dexamphetamine: 0.5-2.0 mg/kg) was studied on the open field surface, using an videotape computer program: HR showed a much higher dose (2.0 mg) HR showed a significantly greater locomotor activity, measured in boxes (30x30 cm) than LR following administration of dexamphetamine at doses (0.5-2.0 mg/kg/g). Second, ethanol consumption was measured. Animals were maintained on alternate day presentation of ethanol and water; ethanol solutions were given in increasing steps of 1%. LR rats showed significantly higher ethanol intake and preference than did HR rats. Animals were maintained on 10% ethanol to determine preference stability; line differences remained stable throughout the entire period. In conclusion HR rats differ from LR rats both in stereotyped and locomotor responses to dexamphetamine and ethanol intake. It remains to be investigated to what extent these differences are causally coupled to differences in the amnestic make-up of the striatum.

240.21

The amphetamine-like abuse potential of dexamphetamine (dFEN) was evaluated using drug discrimination and self-administration procedures. Male Fischer rats were trained to discriminate either dFEN (1.0 mg/kg) or d-amphetamine (dAMP) (1.0 mg/kg) from saline in a two-choice discrete-trial avoidance paradigm. Inamping-trained rats, dFEN (0.5-4.0 mg/kg) engendered almost exclusively saline-appropriate responding. In dFEN-trained rats, dAMP (1.0-4.0 mg/kg) engendered entirely saline-appropriate responding while lower intermediate levels of dFEN-appropriate responding in the remaining animals. Potential reinforcing effects of dFEN were also evaluated in 3 male rhesus monkeys trained to self-administer cocaine (iv) during daily 60 min sessions under a fixed-ratio 10 schedule. Various doses of dFEN (30-1000 ug/kg/infusion) and dAMP (10 mg/kg) were administered for 10 min in four daily daily 60 min sessions. No difference was found between the groups of dFEN maintained rates of self-administration within the range of rates maintained by saline and considerably below those maintained by cocaine and dAMP. Furthermore, the within-session distribution of responding with dFEN resembled that produced by saline. Taken together, these results strongly suggest that dFEN will not have amphetamine-like abuse potential in man.
420.19

FACTORS AFFECTING THE DEVELOPMENT OF BEHAVIORAL SENSITIZATION TO APOMORPHINE. B.A. Mattingsly*, K. Koch, F.H. Gobbel†.
Department of Psychology, Morehead State University, Morehead, KY 40351.

Research support for apomorphine sensitization to the direct dopamine agonist, apomorphine (AP) develops through both associative and non-associative mechanisms. The present study further assessed the role of environmental factors in AP-induced behavioral sensitization. In Exp. 1, rats were treated daily with either AP (5 mg/kg) or vehicle (VEH) and tested for activity in either an open-field or a running-wheel. On Day 9, one-half the rats in each drug/apparatus group were tested in the alternation apparatus. AP-treated rats displayed progressively greater activity over days in both the open-field and running-wheel (F, w. sensitization). In Exp. 2, rats were treated daily with either AP (5 mg/kg) or VEH and again tested in the running wheel. For one-half the rats in each drug condition the wheel was free to move and for the remainder the wheel was immobilized. On Day 10 of testing, all rats received a challenge injection of AP (5 mg/kg) and the wheel was free to move for all rats. AP-treated rats in the free wheel displayed significant sensitization over the 10 test days. In contrast, rats pretreated with AP and placed in the immobilized wheel for 9 days were not sensitized when tested on Day 10. These results suggest that the ability to express the drug-induced locomotor response may be more important than drug-associated cues in the induction of behavioral sensitization.

DEGENERATIVE DISEASE: ALZHEIMER’S—MECHANISMS OF CELLULAR INJURY I

421.1

INHIBITION OF APOLIPOPROTEIN E mRNA TRANSLATION WITH ANTISENSE OLIGONUCLEOTIDES. S. Yajima and M. M. Mournier†.
Genetic Pharmacology Unit, Experimental Therapeutics Branch, National Cancer Institute, Bethesda, MD 20205.

Alzheimer disease patients have increased frequency of apolipoprotein E (ApoE) epsilon 4, suggesting this allele is a risk factor for the disease. ApoE, which interacts strongly with amyloid beta protein in vitro, is immunocohemically localized to the senile plaques, vascular amyloid and neurofibrillar tangles of Alzheimer disease. These new antisense oligonucleotides (ODN) have been synthesized to target apoE mRNA for therapeutic advantage in minimizing amyloid deposition in the disease. The antisense oligodeoxynucleotide (ODN) strategy can be used as an effective tool to evaluate the expression of apoE specific genes. In our investigation, we used phosphorothioate antisense ODNs to down-regulate ApoE gene expression. An expression vector was constructed containing the M. nemestrina, the non-parallel restriction fragment of the human ApoE cDNA fused, in frame, with the reporter gene encoding luciferase and used to generate stably transfected CHO cells. A series of 21 mer antisense ODNs targeted to various portions of the ApoE message were used. Nonsense ODNs having the same nucleotide composition but in scrambled sequence were used as controls. Among the tested ODNs, two molecules spanning the ATG codon added to the culture medium at 50 µg/ml for 24 hours decreased luciferase activity by about 20% (p<0.05). Shorter time points and a lower concentration (µg/ml) were ineffective. Nonsense ODNs to the cultured cells were noted. These findings suggest that the antisense approach could be useful to study the potential pathogenetic link between ApoE and Alzheimer disease.

421.3

APOLIPOPROTEIN E, ALLELE ASSOCIATION WITH ALZHEIMER’S DISEASE. S.E. Poduslo*, J.D. Schwandtshas.
Dept. of Neurology, Texas Tech University Health Sciences Center and Dept. of Veterans Affairs, Lubbock, TX 79430.

The apolipoprotein E, allele has been strongly implicated in late onset familial and sporadic Alzheimer’s Disease (Saunders, et al. Neuro 43:1467, 1993). The authors found the E4 allele frequency to be 0.42 for late onset Alzheimer’s patients, compared to 0.25 in controls. In earlier reports, a higher frequency of E4 allele was noted in patients with Parkinson’s disease (Hukkanen, et al. Neuro 43:1467, 1993). The apolipoprotein E, polymorphism was determined after PCR amplification of genomic DNA, restriction enzyme digestion with HhaI, and polyacrylamide gel electrophoresis. Ethidium bromide stained bands at 91 bp were designated as allele 3, 63 bp as allele 2, and 26 bp of 123 bp as allele 1. The frequencies for the E3 allele in the late onset familial or sporadic patients was approximately 0.4; while that for early onset sporadic patients was 0.29 and for familial was 0.64. We analyzed 177 cases, of whom 12 had the 3/3 genotype and 15 had at least one E4 allele for an E4 frequency of 0.097. In a survey of 53 Parkinson’s patients as another neurological control group, only 9 had the E4 allele. Our findings support the association of the E4 allele with Alzheimer’s Disease. Supported by the State of Texas DNA Bank for Genetic Studies of Alzheimer’s Disease.

420.20

EFFECTS OF WITHDRAWAL FROM NICOTINE ON INTRACRANIAL SELF-STIMULATION. M. Legault* and R.A. Wise.
Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, QC, CANADA H3G 1M8.

Elevated intracranial self-stimulation (ICSS) thresholds following withdrawal from repeated treatments with cocaine, amphetamine or morphine are consistent with a model of depression of the dopaminergic transmission. We investigated whether relevant drug treatments that characterize human drug dependence. Dysphoria is also a feature of nicotine dependence and we now report the effect on ICSS of nicotine withdrawal. Animals were repeatedly injected with 0.5 or 1 mg/kg of nicotine or saline. Injections were given once on the first day of treatment then twice for the next 13 days (equating a dose of 1.0 or 3.0 mg/kg/day) and once on the next day (15). Two measures of rate-frequency threshold were determined every 4 hours for 32 hours following the last injection. Nicotine withdrawal caused non-parallel rightward shifts in the frequency functions. Peak effects occurred between 20 and 24 hours following the final injection. During this time period threshold determined by the lowest frequency required to reinstate responding was elevated to approximately 120% of baseline in each nicotine group, whereas the peak elevation in the frequency required to maintain half-maximal responding was only 10%. These data suggest that repeated nicotine treatment results in a dependence syndrome characterized by a phasic depression of the neural substrate underlying ICSS that is detectable at moderately reinforcing stimulation frequencies.

421.4

Dept. of Neurology, Radium, University of Clinical Genetics, Univ. and Hospital of Kuopio, Kuopio, FINLAND.

Several studies have indicated apolipoprotein E (apoE) e4 as a risk factor for late-onset sporadic and familial Alzheimer’s disease (AD). AD is characterized by senile plaques, particularly amyloid beta (apoE) deposits, and temporal lobe structures. Previously, apoE was implicated in the regeneration of the nerve cell body and in the synaptogenesis of the hippocampus in experimental models. Here, we studied apoE and apoE e4 in 28 AD patients and in 28 elderly controls. The frequencies of apoE e4 were 0.33 in AD patients and 0.24 in controls. The frequency of apoE e4 allele in the late onset Alzheimer’s Disease patients (all of whom were Caucasian), 45 had the 3/4 genotype, 13 had the 4/4 genotype, and 4 had the 2/4 genotype. There were 25 early onset patients and 58 late onset patients. The frequencies for the E3 allele in the late onset familial or sporadic patients was approximately 0.4; while that for early onset sporadic patients was 0.29 and for familial was 0.64. We analyzed 177 cases, of whom 12 had the 3/3 genotype and 15 had at least one E4 allele for an E4 frequency of 0.097. In a survey of 53 Parkinson’s patients as another neurological control group, only 9 had the E4 allele. Our findings support the association of the E4 allele with Alzheimer’s Disease. Supported by the State of Texas DNA Bank for Genetic Studies of Alzheimer’s Disease.

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421.5 ALZHEIMER’S MECHANISMS OF CELLULAR INJURY

APOLIPOPROTEIN E4 AND E4-INDUCED DIFFERENCES IN NEURITE OUTGROWTH ARE ASSOCIATED WITH DIFFERENCES IN THE SUBCELLULAR LOCALIZATION OF APP, AVPR, AND HSPG IN NIGROSTRIATAL REGION

DEGENERATIVE INFECTION

BY W. B. Nathan,* S. Belot,* R. W. Mahley,* and R. E. Plata,* Gladstone Institute of Cardiovascular Disease, Cardiovascular Research Institute, Departments of Medicine and Pathology, University of California, San Francisco, CA 94141-9100.

Apolipoprotein (apo) E4, one of the three common isoforms of apoE, has been implicated in Alzheimer’s disease. Previously, we demonstrated detrimental effects of apoE3 and apoE4 on neurite outgrowth in cultures of dorsal root ganglion (DRG) neurons: In the presence of β-migrating very low density lipoproteins (β-VLDL), apoE3 increased neurite extension and decreased transcellular outgrowth (Nathan et al., Science 1994, 264:850-852). In the present study, we examined the effects of apoE3 and apoE4 on neurite outgrowth from a murine neuroblastoma cell line (Neuro-2a). The products of Neuro-2a medium for 48 hours in the presence of β-VLDL (40 μg cholesterol/ml) alone or with apoE3 or apoE4 (30 μg/ml) purified from human plasma. Consistent with the results obtained with the DRG neurons, Neuro-2a cells increased with apoE3 but decreased with apoE4 as compared with that seen in the cells grown in medium containing β-VLDL alone. Immunocytochemical localization of apoE4 in the Neuro-2a cells treated with apoE3 or apoE4 with β-VLDL revealed a differential localization of apoE. In apoE3-treated cells, intense apoE immunoactivity was observed within the cell bodies and neurites. In contrast, apoE4-treated cells showed little, if any, immunoactivity within the neurites. The data suggest that the localization of apoE3 (but not apoE4) in the neurites may play a role in enhancing neurite outgrowth.

421.7 MICROVASCULAR PATHOLOGIC CHANGES IN HIPPOCAMPAL INFECTION IN ALZHEIMER′S DISEASE: B. Levey**,* C. A. Gooddy,** and R. M. Eilert,** Departments of Neurology and Neurobiology, Mount Sinai Med Ctr, New York, NY 10029; Dept of Psychiatry, RGP Bi-Alt, Univ of Geneva Sch Med, Switzerland.

Biphasic ischemic/hypoxic injury is a major component of the endothelial cell surface and extracellular matrix, plays a fundamental role in the integrity of the blood-brain barrier. Using an antibody to vascular HSPG (7612), we recently described distinct patterns of changes of cerebral capillaries in various regions of the cerebral cortex in Alzheimer’s disease (AD). In addition, a decrease of the immunostaining was observed in all of the cortical areas in AD cases when compared with control cases. Although the endothelial cortex is particularly affected in AD, very little is known about pathological vascular changes in this area. Using two different antibodies to vascular HSPG (7612, EPO1), we investigated the alterations of the microvasculature in the entorhinal cortex of 12 AD cases and 13 elderly control cases. Similar staining patterns were obtained with the two antibodies. Interestingly, no changes of the microvasculature were observed in the control cases in comparison with AD cases suggesting that an overexpression of vascular HSPG occurs in AD. In addition to the microvasculature, some senile plaques and neuritic tangles were also labeled in AD cases. The laminar distribution and relative densities of the microvessels showed a correlation between angio- genetic and neocortical abnormalities. In particular, vascular density was higher in layer II of the entorhinal cortex and in the paraventricular layer of the subiculum and subiculum. Numerous morphological abnormalities such as string vessels and distorted vessels were observed in AD cases. The control cases exhibited some pathological changes, however, to a much lesser degree than AD cases. Preliminary data suggest that a decrease in the vascular density occurs in the hippocampal formations with AD. In conclusion, the morphological and biochemical changes that were observed in the microvasculature of AD brains may profoundly affect the properties of the blood-brain barrier and cause detrimental effects to specific neuronal populations.

421.9 MHC CLASS II ANTIGEN EXPRESSION BY MICROGlia AFTER DEAFFERENTATION IN AGED RATS: M. G. Gordon,* L. A. Holmes, W. A. Scheirer and D. O. Marrag, Dep. of Pharmacology, Univ. South Florida, Tampa, FL 33612-4799.

To examine microglial reactions after brain injury in the aged rat, 6-hydroxydopamine (6-OHDA; 8 μg) was injected into the right medial forebrain bundle of male rats 47 weeks old. Rats were killed 4, 7, 14 or 21 d after lesioning. Unlesioned rats served as controls. Microglial cell number and staining intensity were evaluated by morphometric videoelectronometry. Sections stained immunocytochemically for tyrosine hydroxylase (TH) confirmed the virtually complete loss of nigrostriatal TH in all age groups. Stained sections stained for the microglial specific markers MHC class II antigen (MHC-II; OX6) and complement receptor 3 (CR3: OX42) indicated that MHC-II is induced by the lesion, while CR3 is not. In addition, the MHC-II induction increases with age and duration progressively with age.

421.10 DISTRIBUTION OF MICROGlia IN NORMAL AND ALZHEIMER′S DISEASE (AD) BRAIN SUPPORTS MICROGlia INVOLVEMENT IN NEURONODEGENERATION. L. Q. Shephard* and N. E. Berman, Dep. of Anatomy & Neurology, Univ. of Kansas Med. Ctr, 3901 Rainbow Blvd., Kansas City, KS 66160-7400.

Microglia are the macrophages of the central nervous system and are activated in response to trauma, infarcion, infections, and inflammatory events. With AD, microglial cells are known to be activated with a concomitant phagocytic debris and secrete factors which may be cytotoxic. In Alzheimer′s disease (AD), microglial cells have been found in direct association with neuritic plaques (NP), one of the hallmarks of AD. The region specific localization of neuritic tangles (NFTs) and NPs is used to diagnose AD in postmortem brain tissue. In early stages of AD, NFTs are found in the entorhinal region of the parahippocampal gyrus. As the disease progresses, NFTs are also found in limbic structures and eventually in selected neocortical areas, but are almost entirely excluded from the primary motor cortex.

Our studies have focused on the distribution of microglia in both normal and AD brains. Microglia were visualized by lectin histochemistry using the lectin Ricinus communis agglutinin-1 (RCA-1). In control brains, the density of microglia was greater in the regions which are most severely affected by AD, i.e., the parahippocampal gyrus (entorhinal)striate. The region area 22 exhibited a much lower density. In AD brains microglial density increased in all areas of the brain with longer periods of dementia, but the pattern of distribution seen in control brains was not constant. In AD brains, microglia are found in neuritic plaques, neuritic tangles and NFTs in the parahippocampal gyrus, where the majority of AD pathology is concentrated.
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WEDNESDAY AM

421.11 
QUANTIFICATION OF THE IL-1B INDUCED MESSAGES, PLASMINOGEN ACTIVATOR INHIBITOR TYPE-1 AND PROSTAGLANDIN GH SYNTHASE-2, IN ALZHEIMER'S DISEASE. J.W. Chang, D.J. Selvik, P.D. Coleman, and M.K. O'Driscoll, Departments of Neurobiology and Anatomy and Neurology, University of Rochester School of Medicine, Rochester, NY 14642. 

In response to CNS injury or neurodegenerative disorders, cytokines such as interleukin-1 (IL-1B) are released in the microenvironment. One role of IL-1B is to activate astrocytes and to increase the synthesis/secretion of astrocytic proteins. We have previously demonstrated that IL-1B promotes astrocytes to secrete plasminogen activator inhibitor-1 (PAI-1), which may influence neurogenesis, and synthesize prostaglandin GH synthase-2 (PGHS-2), an inflammatory protein. The levels of PAI-1 and PGHS-2 may serve as markers for astrocyte activation by IL-1B. Since IL-1B levels are elevated in Alzheimer's disease (AD) brains, we hypothesized that PAI-1 and PGHS-2 mRNA may be increased in AD brains relative to age-matched controls. 

We carried out Northern hybridization of poly A mRNA from the putamen and subcortical area (necrotic area ventral to genu of corpus callosum) of AD and age-matched control brains utilizing cDNA probes for human PAI-1, PGHS-2, and G3PDH. Hybridization signals, quantified using a Molecular Dynamics PhosphorImager, were normalized using G3PDH signals. Our preliminary data indicate little or no alteration in PAI-1 and PGHS-2 mRNA levels with disease in the putamen (N=5 AD cases, 4 controls). However, in the subcortical area (Brodmann's areas 12/52), PAI-1 and PGHS-2 mRNA levels are decreased 1.5-fold and 3-fold, respectively, between AD (N=3) and age-matched control (N=5) brains. 

These observations are somewhat surprising given the detection of IL-1B in AD brain. Further studies in other affected brain regions are in progress. [Supported by LEAD award G009018, R01 AG11121, and training grant T32 AG1017] 

421.12 
HUMAN ASTROCYTES CAN EXPRESS COMPLEMENT C4 mRNA AND SECRETE COMPLEMENT C4 PROTEIN. D.G. Walker*, S.U. Kim* and P.L. McGeer†. *Kinsmen Lab. of Neurological Research, Department of Psychiatry, 1Division of Neurology, Department of Medicine, University of British Columbia, Vancouver, B.C. Canada, V6T1Z3. 

Studies of brains from Alzheimer Disease (AD) cases have shown evidence of inflammatory changes in affected tissue, as demonstrated by complement system activation and the presence of inflammatory cytokines. These features indicate that a chronic inflammatory response occurs in AD brains which may be contributing to the neurodegenerative process. In addition, a pronounced astrocytic glial response is evident in AD tissue. There is now increasing evidence that astrocytes may be contributing to the inflammatory changes by the production of certain cytokines and complement proteins. 

In this study, we show that astrocytes isolated from formalin fixed AD brains, and cultured in vitro, can express the mRNA for complement C4, and can also secrete the complement C4 protein. Using the reverse transcription-polymerase chain reaction (RT-PCR) technique, the expression of C4 mRNA was shown to occur in six separate sets of cultures that were highly enriched for astrocytes (>95% of cells immunoreactive for glial fibrillary acidic protein). Expression was detected in unstimulated astrocytes and this was increased by treatment of cells with LPS interferon (100 or 200 units/ml of recombinant L interferon). In addition, by culturing the cells in serum-free media and by using immunomodulating and IL-1α techniques, we showed that human astrocytes can secrete C4 protein into the culture media. Increased secretion of C4 occurred after treatment of astrocytes with L interferon, but not with interleukin 1 (20 or 200 units/ml). 

Supported by a grant from the British Columbia Health Research Foundation. 

421.13 
DOUBLE IMMUNOLABELING OF COMPLEMENT PROTEINS AND NEUROFIBRILLARY TANGLE MARKERS AT LIGHT AND ELECTRON MICROSCOPIC LEVELS IN ALZHEIMER'S DISEASE BRAIN. L.-F. Lee*, S. Webster†, S. Greenberg*, J. Rogers†, Sun Health Research Institute, Dept. of Neurology, Room C5, Tucson 85724 and Burke Medical Research Institute, White Plains, NY 10605. 

A pathogenic role of complement activation in Alzheimer’s disease (AD) has been suggested by recent evidence that the complement cascade is activated at sites of amyloid β peptide (Aβ) deposition through Aβ/C3b mediated binding. This process also appears to accelerate aggregation of soluble Aβ into its pathogenic fibrillar β-plated conformation. However, activated complement components C4, C5, C3d, and C3b also co-localize with cells exhibiting neuronal morphology, particularly neurones containing neurofibrillary tangles (NFTs). In this study, we show that the levels of complement immunoreactive NFTs containing tangles in cortical samples from four pathologically confirmed AD patients. Light and electron microscopic examination of sections of these brains confirmed that the majority of the L immunoreactive cells in AD brain contain NFTs. Taken together with certain features of Aβ/C3b binding and binding sites, these data suggest that complement activation may occur at the cellular surface of degenerating neurons, with lethal consequences. An interaction with NFT formation is also possible given the mechanism of C5b-9 attack on targeted cells. [Supported by NIA AG3767] 

421.14 
ALZHEIMER’S DISEASE LIKE PHOROLYSIS OF TAU PROTEIN IN RESPONSE TO INTERLEUKIN-1β TREATMENT OF MDM: POPULATIONS FROM RAT CORTIC. S. Wewarl and M.L. Shalem*. Department of Pathology and Alzheimer’s Disease Research Center, Columbia University College of Physicians and Surgeons, New York, NY 10032. 

Immune related processes have been associated with Alzheimer’s disease (AD) as have significant reductions in the prevalence of AD among patients treated with anti-inflammatory therapies. However, a physiological mechanism for the involvement of immune responses in producing AD-like pathology has not been demonstrated. Using antibodies (Abs) to phosphorylation sensitive epitopes of tau, we have demonstrated Abs to the tau protein in response to the inflammatory cytokine interleukin-1β in vitro. 

Mixed cell cultures from postnatal day one (PND-1) rat brain cortex were incubated in serum free medium supplemented with the cytokine interleukin-1β (50 U/ml). Whole cell homogenates were then analyzed by Western blotting with two Abs. IL-1β produced an increase in high molecular weight immunoreactivity to the phosphorylation sensitive epitope of tau (hyperphosphorylated tau specific Abs) and the N-terminal domain of tau (anti-tau Ab was lost upon prolonged incubation. These changes in antigenicity parallel the changes seen in human AD brain. 

Decreased gel mobility of tau isoforms was seen upon IL-1β treatment. Conversely, co-incubation of tissue with IL-1β and increasing amounts of IL-1 receptor antagonist showed a dose dependent increase in tau mobility. These results demonstrate that IL-1β may contribute to the abnormal phosphorylation of tau, which subsequently could decrease the stability of tau-nuclear interactions and contribute to PHF formation. Supported by NS-15076. 

422.1 

Senile plaques (SPs), neurofibrillary tangles (NFTs) and neuritic threads (NTs) are currently studied using low or fluorescent microscopic methods that provide 2-D images of their distribution within tissue sections. Such methods are of limited value in studies of antigen or antibody localization, that the majority of complement- or surface structure identification are important. In theory, confocal laser scanning microscopy (CLSM) provides one means to identify the 3-D location of structures within specimens. To evaluate this, the morphology and distribution of SPs, NFTs and NTs were examined in the brain of one 75-yr old patient with AD. Frozen tissue sections (3-30 μm) were cut from the hippocampus and entorhinal cortex and were fixed with 3% glutaraldehyde (Sheriden et al., 1991). Serial sections (Z axis increment: 0.5-1.0 μm) of individual SPs, NFTs and NTs (n=20) were generated with CLSM, resulting in a composite image. The spatial dye. SPs, NFTs and NTs on the surface were easily distinguished from those buried deeper within the section. The results demonstrate that CLSM provides a basis for determining the penetration of reagents in tissue staining and is useful as a guide for surface location of trace element distributions using IMS.
422.3

Calpains are believed to be important regulators of membrane dynami-
ces and membrane trafficking, but mediate neurodegeneration in certain path-
ological states. We previously reported evidence for decreased activa-
tion of the calpain-calpastatin system in Alzheimer brain. Also, calpastatin, the specific protein inhibitor of calpain, was shown by immunocytochemistry to be markedly depleted in Alzheimer neurons (Nixon et al., Soc. Neurosci. 1998, 1419-1992). To confirm this biochemically, we measured calpain levels in homogenates of prefrontal cortex from 13 Alzheimer patients and 9 normal controls matched by age and postmortem interval. Calpastatin immunoreactivity and inhibitory activity were measured in soluble and membrane fractions. In normal human brain, membrane fractions solubilized by Triton X-100 contained 40% of the total calpastatin (20 ± 15.6 units/g tissue). Calpastatin was identified by immunoblotting using affinity purified antibodies to be composed of 110, 70 and 41 kDa forms. Calpastatin inhibitory activity in membrane fractions of Alzheimer pre-
frontal cortex was reduced to 58% of control values (p < 0.001). By immuno-
blot analysis the most abundant calpastatin form isolated from human brain (M = 41 kDa) was reduced > 65% in membrane fractions (p < 0.001). Cytosolic calpastatin activity was 21.9% lower (p < 0.005) but major immunoreactive forms were markedly reduced (75.6, p < 0.01) reflecting proteolytic processing to smaller molecular weight inhibitory units. Calpastatin was not significantly altered in cerebellum. These results, together with the abnormal activation of γ-
calpain reported earlier (Saito et al., PNAS 99:2628, 1993), implicate calpains in the disruption of membrane dynamics possibly leading to altered membrane protein processing and neurodegeneration in Alzheimer Disease. Supported by NIA (AG10916).

422.5
IN-VITRO UBQUITINATION AND DEGRADATION OF TAU PROTEINS. R.S. Black* Cornell University Medical College at Burke Medical Research Institute, White Plains, NY 10605.
The concomitantly phosphorylated tau proteins, and the subsequent failure of their ubiquitin-mediated proteolysis is an important feature of the Alzheimer disease process. The DEAE-cellulose fraction of rabbit reticulocyte lysates containing enzymes of the ubiquitin conjugation system (Fraction II) was used to conjugate ubiquitin to tau proteins prepared from twice-cycled bovine brain microtubules. In the presence of ATP, an ATP regenerating system, and hemin, which inhibits the breakdown of ubiquitin conjugates, high molecular weight ubiquitin-conjugated tau proteins accumulated. In the absence of hemin the ubiquitin conjugates did not accumulate and the tau proteins appeared to be degraded in an ATP-dependent fashion. In this system, ubiquitin-ubiquitin conjugates were detectable almost immediately in hemin-inhibited lysates, whereas tau-ubiquitin conjugates were detectable only after 1-2 hour incubations. Bovine MAP-2 was also a substrate for ubiquitin conjugation in this system. This system is being used to evaluate the effects of post-translational modifications of tau proteins on ubiquitin conjugation. Supported by the NIA (AG00504).

422.7
CONSTITUTIVE ALZHEIMER'S-TYPE TAU EPITOPES IN A NEUROTIGenic RAT CNS CELL LINE. M.P. Lambert*, S. Sabo, C Zhang, S.A. Fanan, and W.L. Klein. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Paired helical filaments (PHFs) of Alzheimer's disease (AD) largely comprise hyperphosphorylated forms of the cytoskeletal protein tau. AD-type tau phosphorypates are absent from normal adult neurons, but recent studies have shown that their expression may correlate to neurogenesis and axon differentiation in the developing brain. Therefore, we examined the enrichment of a cell line that is spontaneously neurogenic for possible expression of AD-type tau epitopes. The N110 of CN5 cell line was found to produce constitutively two AD-related epitopes of tau, detected by cellular immunofluorescence with the monoclonal antibodies E1 and Alz-50. Biochemical studies showed that the antibodies bound to proteins within the molecular weight range expected for phosphorylated tau isoforms. Further verification was established by use of tau antisera, which eliminated immunofluorescence due to the AD-related monoclonals and polyclonal anti-tau, but did not eliminate fluorescence due to anti-tubulin. Cells treated with tau antibodies were not neurite free. Neurites removed were abnormal, generally short, and wavy in appearance. Cellular distribution of the tau epitopes was particularly interesting. Alz-50 immunoreactivity was found only in the cytoplasm, while PHF-1 immunoreactivity was found in the nucleus as well as the cytoplasm. Thus the two epitopes are morphologically segregated within the cell. Because subcellular segregation of tau is compromised in Alzheimer's disease, mechanisms that segregate Alz-50 and PHF-1 epitopes in B10 cells may have relevance to this neurodegenerative disorder.

422.4

Tau is a family of microtubule-associate proteins which play a crucial role in the structure and function of the neuronal cytoskeleton. Tau in hyperphosphorylated states also forms the paired helical filaments (PHFs) of Alzheimer's disease. Although the phosphorylation of tau has been extensively delineated, other modifications have not been well defined. Recently, a single modification has been identified at Ser and Thr residues consisting of an N-linked N-acetylglucosamine (O-GlcNAc). This modification is found in a variety of proteins, including neurofilaments. The sites of O-GlcNAc glycosylation are indistinguishable from the phosphorylation sites of Ser/Thr kinases. Purified bovine tau was analyzed using galactosyltransferase as a purifying tool and GlcNAcs, followed by peptide-N-glycosidase F treatment and β-
elimination for linkage analysis. The results of these studies clearly demonstrate that tau is modified by O-GlcNAc glycosylation. This unique form of glycosylation may modulate tau function, and in specific neurodegenerative disorders may contribute to neuronal dysfunction and the formation of pathological lesions. Supported by NIH grants CA42846 (GWH) and NS27538 (GVW).

422.6
THE ABNORMAL PHOSPHORYLATION OF TAU PROTEIN AT SER-202 IS PREFERENTIALLY LOCATED IN NEURITES AND PRECEDES ABNORMAL PHOSPHORYLATION AT SER-396 IN ALZHEIMER'S DISEASE. Joseph H. Ste*, Brian J. Cummings and Carl W. Cotman. JBU in Brain Aging. UCLA, Irvine, CA 92717 USA.

Both neurofibrillary tangles (NFTs) and dystrophic neurites (DNs) contain paired helical filament (PHF) which are composed of abnormally phosphorylated PHF-tau. During formation of NFTs and DNs in AD, the cytoskeleton undergoes a sequence of changes, including hyper-phosphorylation of tau protein (for review, see Trojanowski et al., 1993). We sought to determine more specifically at what tau protein residue the earliest changes take place; and whether these alterations first occur within distal processes or within the soma. We used two monoclonal antibodies, AT8 and PHF-1, which selectively recognize phosphorylated Ser-202 and Ser-396 of PHF-tau protein respectively. Both antibodies stained NFTs and DNs, including dendrites and axons; however the quantity and distribution of immunoreactive deposits was different. AT8 immunoreactivity was usually found within intracellular NFTs as well as extracellular neurites, whereas PHF-1 positive fibrillary inclusions were detected within both intracellular and extracellular NFTs. Some irregularly labeled AT8 DNs were continuous with "normal" neuronal somas in which AT8 immunoreactivity was absent or weak. However, PHF-1 positive DNs were only continuous with PHF-1 positive NFTs. In AD cases with mild dementia, numerous AT8 deposits were detected in the outer two-thirds of the molecular layer of the dentate gyrus, whereas no PHF-1 positive deposits could be found within the same region in adjacent sections. These results suggest that abnormal phosphorylation at Ser-202 of PHF-tau in DNs represents one of the earliest neuropathological changes within the neurites of vulnerable neurons and might play an important role in the initial pathogenesis of AD.

422.8
OKADAIC ACID INDUCES MICROTUBULE DEPOLYMERIZATION AND THE DEGENERATION OF AXONS AND DENDRITES IN THE NTT2 CULTURE SYSTEM: IMPLICATION IN ALZHEIMER'S DISEASE. S.E. Merrick and V.M.Y. Lee. Institute of Neurological Sciences, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA.

The building blocks of Alzheimer's disease (AD) neurofibrillary tangles (NFTs), are paired helical filaments that are comprised of hyper-phosphorylated forms of tau (i.e. PHF-tau) and the accumulation of PHF-tau may be due to an inactivation or down-regulation of brain phosphatase, such as NEP bearing phosphatase. Since exquisitely phosphorylated PHF-tau is unable to bind to microtubules (MTs), then conversion of normal tau into PHF-tau by the inhibition of protein phosphatases could lower the level of tau that binds MT, destabilize MTs, disrupt axonal transport, and lead to the "dying back " of axons in AD. To test this hypothesis, we developed an in vitro neuronal culture model that allows us to examine the consequences of the inhibition of protein phosphatases on the neuronal cytoskeleton. The model is based on neurons (NT2N) derived from a human teratocarcinoma cell line (NT2) that are treated with retinoic acid to induce differentiation and then exposed to okadaic acid (OXA), a potent phosphatase inhibitor. OXA increased tau phosphorylation in NT2N neurons resulting in the inability of high phosphorylated tau to bind to MTs. OXA-induced increased tau phosphorylation, there was an increase in the depolymerization of MTs which was due to the increased conversion of stable Glu-tubulin to MBs to monomeric Tyr-
tubulin. As a consequence of the loss of MBs, there was dying back" of the MTs and the axons of NT2N neurons prior to the complete destruction of dendritic and cellular MTs. Our results suggest that the inhibition of protein phosphatases in neurons leads to the destabilization of the MT network in axons by: 1) Increased tau phosphorylation; 2) A reduction in the number of the tubulin-
lysine ligand that converts Glu-tubulin to MTs free Tyr-tubulin.

The paired helical filaments (PHFs) of Alzheimer's disease (AD) are composed of highly phosphorylated tau proteins. Sites of these sites were found to be phosphorylated in PHF-tau, fetal-tau, but not in adult brain tau. To determine the phosphorylation state at these sites during development, we isolated tau from fresh rat brain in the presence of the phosphatase inhibitor okadaic acid (OK) to obtain tau in its native state of phosphorylation in situ. Fetal tau immunoreactivity was reduced by 50% by 18E11-directed post-natal day 11 (P11) after which the immunoreactivity diminished concomitant with the decrease in phosphorylation and the appearance of the mature isoforms. In contrast to previous studies, adult and aged rats (20 months) were found to be phosphorylated at reduced levels suggesting that Thr^218, Ser^202, Thr^231, Ser^235 and Ser^404 are normal sites of phosphorylation in adult rats. We also show that inactivation of OK in the assay buffer has a transient effect on the abilities of tau to bind MTs at any developmental stages. However, an activation of phosphorylation at these sites was absent when done in the absence of OK tau was shown to be partially dephosphorylated. We further demonstrate that protein kinase 2A (PKA) and 2B (PKB) from the adult rat brain could dephosphorylate tau efficiently in a site specific manner, whereas protein kinases from P6 had no effect indicating that OK sensitive tau after P12 may be regulated by the de novo induction of adult brain phosphatases. Collectively, these findings suggest that PKA-like phosphatases are involved in regulating the adult phosphorylation state of tau in-vivo, and they open the possibility that the generation of hyperphosphorylated tau leading to PHF formation in AD may be controlled by similar phosphatases.

422.12 ALZHEIMER TYPE PHOSPHORYLATIONS OF TAU PROTEIN ARE DEVELOPMENTALLY EXPRESSED IN VITRO. C.K. Combs*, P.O. Coleman, and M.K. O'Brien. Departments of Neurobiology and Anatomy and Neurology, University of Alabama at Birmingham, Birmingham, AL 35242.

It has been shown that certain Alzheimer type tau phosphorylations occur embryonically in humans and postnatally in rats. Determining the nature of the regulatory mechanisms of these phosphorylations in these events may provide a better understanding of the aberrant phosphorylation of tau occurring in Alzheimer's Disease.

We have utilized embryonic rat hippocampal cultures to investigate the expression of specific Alzheimer type tau phosphorylations during in vitro differentiation. The mouse monoclonal antibodies to PHF-1 and PHF-2 recognize a phosphorylation on Ser^396 and the lack of phosphorylation on Ser^393, respectively. Using PHF-1 and Tau-1 we have observed a temporal expression of phosphorylation by immunoblotting and Western blot analyses. Phosphorylation at the PHF-1 epitope is observed at the earliest time point in vitro (i.e. embryonic day 16). In contrast, phosphorylation at the Tau-1 epitope was detected around day 4 in vitro and appears to increase over the two weeks examined.

The temporal expression of these phosphorylation events suggests different regulatory mechanisms for each. Future investigations with this in vitro developmental system may not only define the kinases and/or phosphatases involved, but may also provide clues about the physiological roles of these phosphorylation events. (PHF-1 and Tau-1 Abs were obtained from Peter Davies and Lester Binder, respectively) [Supported by LEAD award A005016, RO1 AG01121 and training grant T32 AG10147].


Supply of excess ATP to isolated brain microtubules induced Alzheimer type phosphorylation of tau proteins, providing a potential model to study Alzheimer tau phosphorylation and regulation. Although immunoblot analysis indicated that MAP kinase and CDK are present in microtubules obtained by purification and that purified MAP kinase and CDK increased the level of tau phosphorylation induced by excess ATP, the addition of CDK 1/cyclin B to microtubules partially reverses this type of tau phosphorylation. Furthermore, ATP induced tau phosphorylation is inhibited by treatment of microtubules with apolipoprotein E. The effect of apolipoprotein E is specific and dose dependent. Inhibition of Ca^2+ calmodulin-dependent protein kinase and cAMP-dependent protein kinase have no profound effects on the phosphorylation of tau or on the generation of tubulin from microtubules using taxol abolishes ATP induced tau phosphorylation, suggesting that tubulin may be involved in the regulation of tau phosphorylation by signaling proteins associated with microtubules.AG 11123, NS 27847, NS 30435.

422.14 ALZ-50 AND PHF-1 IMMUNOREACTIVITY NEAR 3-AP INJECTIONS IN THE CEREBRAL CORTEX OF SHEEP ET, Nelson and C.B. Jaing, Beth Israel Hospital and Harvard Medical School, Boston, MA 02115.

We have recently discovered that neurons in the cerebral cortex of sheep and goats develop Alzheimer-type neurofibrillary degeneration (NFD) in the absence of beta-amyloid peptide (3-AP) deposition during aging. Hence, these domesticated animals may provide a novel model for studying the mechanism of induction of Alzheimer-type NFD.

In an effort to induce experimental neurofibrillary pathology in vivo, we injected 3-AP (1ml) into the cerebral cortices of two five-year-old sheep. Following one or two weeks of survival, brains were fixed at 3000g's and sections were stained with toluidine blue and xylene. The 3-AP induced cytoplasmic and nuclear protein A50 was present in some areas of the sheep brains. In addition, these sections were stained with toluidine blue and xylene. The 3-AP induced cytoplasmic and nuclear protein A50 was present in some areas of the sheep brains. In addition, these sections were stained with toluidine blue and xylene.

In the animal that survived one week, the 3-AP injections caused necrosis. Some A50 or PHF-1 immunoreactive dystrophic neurites were seen in direct apposition to the injections within deep cortical layers. However, near the cortical surface, some A50 or PHF-1 immunoreactive neurites appeared that were more swollen than normal immunoreactive neurites. A50 immunoreactive neurites, when viewed via electron microscopy, contained large vesicles. In the animal that lived for two weeks, similar staining was seen, except that some A50 immunoreactive neurites had infiltrated the injection regions.

The current study indicates that, under the conditions we employed, injection of 3-AP does not induce degenerative response in which modified tau proteins are present in neurites.

The localization of apolipoprotein E (ApoE) in relation to beta-amyloid (B440) and paired helical filament (PHF) immunoactivity was examined in the neocortex basal nuclei (NBM), amygdala (AMY), and entorhinal (EC) temporal and insular cortices of patients with AD (n=8) and Down’s syndrome (DS, n=3), age matched normals (AMC, n=8), and age matched nondemented cases with numerous senile plaques (HPND, n=5). ApoE immunostained plaques, neurons, and extracellular tangles in each group. ApoE’s immunostaining of neurons differed dramatically from PHF depending upon brain region and diagnosis. In the AD and DS brain, numerous ApoE-IR neurons closely matched PHF in distribution and quantity in the NBM, AMY and EC. Although PHF-IR temporal and insular neurons were similar in density to the AMY and EC, ApoE-IR neurons were 4-fold fewer than PHF-IR neurons. In the HPND cases, many PHF-IR neurons were observed in the NBM, AMY and EC but were less than in AD. In contrast to these same regions in AD and DS, very few ApoE-IR neurons were observed relative to PHF. These findings suggest that PHF immunoreactivity precedes that of ApoE indicating ApoE may be binding to tangles only in their later stages of formation. Since the HPND cases ApoE recognizes only a few of the PHF-IR neurons in the NBM, AMY, and EC, which are among the earliest brain regions affected in AD, it is possible that ApoE plays a secondary role in tangle formation. Supported by NIH grant AG10161-03.


Neurofibrillary tangles (NFT) composed of paired helical filaments (PHF) are the most characteristic histopathological feature of Alzheimer’s disease. Recent molecular and biochemical studies have provided conclusive evidence that the major integral protein in an abnormally tau protein that is hyperphosphorylated, referred to as PHF-tau. PHF-tau antibodies are present in NFT within the cytoplasm of living neurons (intracellular NFT) and also in NFT in the extracellular space after the neuron with NFT dies, so-called extracellular NFT (EFN). Epitopes in the amino half of tau protein (e.g., Alz50 epitope) are susceptible to proteolysis and are lost in NFT. Neurons in lamina II of the entorhinal cortex (EC-II) are unusually susceptible to NFT, while those in the dentate gyrus are unusually resistant to NFT. By the time AD is advanced, most of the NFT in the entorhinal cortex are NFTe, while most of the NFT in the dentate gyrus are NFTp. Using their distribution in the hippocampus as an index to their most probable type and double-labeling immunocytochemistry of cryostat or frozen vibratome sections, we further characterized NFT in AD. Monoclonal antibodies to NFT that do not cross-react with tau proteins (Ab39 and Ab69) were used as a marker for both NFTe and NFTp, while Alz50 served as a marker for NFTp. NFT, but not NFTe, were labeled by monoclonal and polyclonal antibodies to p34γtubulin. The anti-p34γtubulin antibodies also labeled granular cytoplasmic and perinuclear structures consistent with so-called “pre-tangles”. Monoclonal and polyclonal antibodies to apolipoprotein-E labeled primarily NFTe. NFTe were also inconsistently labeled with antibodies to C4q, Aβ and ubiquitin. These results suggest that in early formative stages NFT are composed of abnormally tau protein in close association with kinases, such as p21akt, that form of phosphorylating tau and that only in later stages of evolution, after the neuron has died, is there substantial association with apolipoprotein-E, Aβ and C4q. (Supported by NIA grants AG08603, AG10136 & AG09145.)

NEUROFIBRILLARY TANGLE NEURONS IN ALZHEIMER’S DISEASE ARE ASSOCIATED WITH A LOSS OF SYNAPTOPSIS MESSAGE. (L. M. Callahan*, J.E. Cheetham, W. Yen, and P.D. Cefaloni.) University of Rochester Medical Center, Neurobiology and Anatomy, Rochester, NY 14642.

Recent evidence indicates the best correlate with the degree of dementia of AD may be the loss of synapses (e.g., DeKosky and Scheff, 1989, Terry et al., 1990). We hypothesized neurons containing tangles lose synapses due to the cytoskeletal disruption of the neuron. To investigate this possibility, we combined immunocytochemistry to identify tangle-containing neurons with in situ hybridization for selected messages. Message levels of synaptophysin, poly A, and catepsin D were determined in serial hippocampal sections from 3 AD cases and 2 age-matched controls. A decrease in the level of synaptophysin, a synaptic vesicle associated protein, was detected in a majority of tangle-bearing neurons relative to levels in neighboring non-tangle neurons. Poly A (total) message levels were similar in many tangle-bearing neurons relative to non-tangle neurons. The demonstration of decreased message level for a protein related to synaptic function in NFT neurons would indicate that it is the NFT neurons that are correlated with synaptic loss in AD. (Supported by NIH AG00107, NIH AG01121, NIH LEAD AWARD AG09016, Alzheimer’s Disease Center Grant NIH AG08665, and the American Health Assistance Foundation).


The hippocampal region including the dentate nucleus is, for the most part, spared in the early stages of AD. However, in the advanced stages, the dentate system is usually affected. The dentate nucleus is an active site of neurogenesis and produced many new neurons. It is thought that Dexamethasone is a neuroprotective agent against the development of AD. In the present study, we examined the effect of Dexamethasone on the dentate nuclei of the rats with experimental Alzheimer’s disease. We studied a total of 8 rats, 4 treated with Dexamethasone and 4 untreated. The rats were treated every day with 2mg/kg body weight for 28 days. We examined the numbers of new neurons, the volume of the dentate nuclei and the weight of the dentate nuclei. The results showed that the number of new neurons increased significantly in the rats treated with Dexamethasone. The volume of the dentate nuclei and the weight of the dentate nuclei also increased significantly in the rats treated with Dexamethasone. These results suggest that Dexamethasone can protect the dentate nuclei from the development of AD.

AUTODETECTION AND LARGE-AREA MAPPING OF NEUROFIBRILLARY TANGLES IN ALZHEIMER’S DISEASE. L.S. Hibbard*, D.W. McKee Jr., Joseph L. Price, Departments of Neurology and Neurological Surgery, Pathology, and Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Neurofibrillary tangles (NFT), seen in normal aged and Alzheimer’s disease (AD) brains, are inhomogeneously distributed in brain. NFT anatomic distributions and densities have been shown to correlate with clinical dementia severity. To quantitate NFT deposition, we have developed computer programs which automatically locate NFT in digital micrographs of postmortem tissues stained with either the Gallyas silver stain or one of several immunostains (anti PHF, anti-tau). Stained objects are detected by template correlation and extracted as discrete objects whose gray level and morphometric properties are used for classification and characterization. The NFT are sorted from all other detected objects using a conventional Bayesian classifier. NFT can be mapped over arbitrary, large areas of tissue, imaged as mosaics of adjacent, non-overlapping, digital microscope fields. Arbitrary regions of interest (ROI) are sketched, using computer graphics, in images of the entire mosaic at low magnification. The ROI guides subsequent processing to only those parts of fields coincident with the ROI. This method can map PHF/tau-immunoreactive cells in very mildly demmented or normal aged brains. (Supported by NIH 5PS0-AG05681)
ISCHEMIA: MECHANISMS I

432.1 THE EFFECTS OF IN VITRO ISCHEMIA ON PROTEINS FROM THE RAT HIPPOCAMPAL SLICE. J. Murua and K.M. Fallis-Syman*. Dept. of Biology Vassar College, Poughkeepsie NY 12601.

Our previous data that total protein synthesis is dramatically inhibited following a 5 min episode of in vitro ischemia in neurons in the rat hippocampal slice. In the present report, we explored the responses of individual proteins to this injury. Approximately 3000 µm slices from male Sprague-Dawley rats and incubated them in a Ringer's buffer equilibrated with 95% O2:5% CO2 at 37°C. Following an ischemic episode period, experimental slices were exposed for 5 min to Ringer's lacking glucose and oxygen. Slices were allowed to recover for 30 min, 1 hr, 2 hr or 3 hr, then were moved into ice-cold homogenization buffer containing 0.32 M sucrose, 10 mM Hepes, 10 mM MgCl2, 15 µg of total protein, and the Bradford protein assay was loaded onto a 7.5% polyacrylamide gel and was separated in one-dimensional gel according to Laemmli. Some gels were silver-stained and analyzed by densitometry. Some gels were transferred to nitrocellulose membranes and exposed to antibodies against hsp-72/73, GFAP, MAP-2 or calbindin-D-28k. In some experiments, new protein synthesis was determined by exposing slices to 35S-methionine and silver-stained gels were processed for autoradiography. Immunoblotting and autoradiograms were also analyzed by densitometry.

In in vitro ischemia inhibited new protein synthesis of most proteins analyzed. The total amount of some proteins was reduced at 2 hr postischemia, while that of others was unchanged. The most notable exception was the response of hsp-72/73 in to ischemic slice. This protein showed a dramatic increase in new synthesis 30 min and 1 hr after ischemia, followed at 3 hr by a 50% decrease in new synthesis. At 2 hr following the insult, the total amount of hsp-77/73 was significantly increased in postischemic slices, when compared with control slices. Thus, while total amounts of protein remains unchanged following ischemia and total new synthesis is reduced, the responses of individual proteins to ischemia must be considered. These individual responses could play an important role in the response to ischemia.

432.2 BRIEF PRECONDITIONING HYPOXIA PROTECTS HIPPOCAMPAL NEURONS FROM SUBSEQUENT HYPOXIA-INDUCED DAMAGE VIA MECHANISMS REQUIRING ONGOING PROTEIN SYNTHESIS. J. Gage* and P.K. Wood*.

Department of Neuroscience & Neurology, Albert Einstein Coll. Med., Bronx, NY 10461

Pre-exposure of rat hippocampi to a short period of hypoxia increases the resistance of CA1 pyramidal neurons to a normally fatal hypoxic insult. We hypothesized that this protective effect is that a short hypoxic insult causes the synthesis of new proteins that protect neurons from subsequent more severe hypoxic insults. We tested this idea by using the reversible protein synthesis inhibitor cycloheximide (CYCLO) and determined if it prevented the priming neuroprotection during hypoxia. We used a biphasic stimulation protocol consisting of the brief preconditioning ischaemia with the Bradford protein assay, was loaded onto a 7.5% polyacrylamide gel and was separated in one-dimensional gel according to Laemmli. Some gels were silver-stained and analyzed by densitometry. Some gels were transferred to nitrocellulose membranes and exposed to antibodies against hsp-72/73, GFAP, MAP-2 or calbindin-D-28k. In some experiments, new protein synthesis was determined by exposing slices to 35S-methionine and silver-stained gels were processed for autoradiography. Immunoblotting and autoradiograms were also analyzed by densitometry.

In in vitro ischemia inhibited new protein synthesis of most proteins analyzed. The total amount of some proteins was reduced at 2 hr postischemia, while that of others was unchanged. The most notable exception was the response of hsp-72/73 in to ischemic slice. This protein showed a dramatic increase in new synthesis 30 min and 1 hr after ischemia, followed at 3 hr by a 50% decrease in new synthesis. At 2 hr following the insult, the total amount of hsp-77/73 was significantly increased in postischemic slices, when compared with control slices. Thus, while total amounts of protein remains unchanged following ischemia and total new synthesis is reduced, the responses of individual proteins to ischemia must be considered. These individual responses could play an important role in the response to ischemia.


Brief ischemia induces tolerance to subsequent more severe insults. Preferential induction of ischemia-resistant neurons in vulnerable hippocampal neurons after brief ischemia suggests that the stress response could contribute to ischemic tolerance. Temperature during early recirculation can significantly alter the survival of ischemia-resistant neurons in the gerbil as well as the expression of hsp72. We have used the temperature dependence of hsp72 expression to assess its role in ischemic tolerance. Gerbils were subjected to 2 min bilateral carotid artery occlusion followed by 90 min recirculation under halothane anesthesia, during which temperature was either maintained continuously at 37°C (normothermic, NT) or elevated to 39.5°C between 15 and 60 min recirculation (hyperthermic, HT). A 5 min ischemia was produced 2 d after the priming challenge, and injury to CA1 was assessed 7 d after this test insult. In other animals hsp72 induction was evaluated by in situ hybridization 3 h after 2 min ischemia in the NT and HT groups. Control neuron density (295±11) was severely reduced after 5 min ischemia in naive animals (1664±180), and striking protection was observed in both NT and HT pretreatment groups (191±96 and 92±101, respectively). Reduced cell density in the HT group reflected a modest loss seen with the priming insult alone. Most importantly, 90% of 2 min NT animals showed significant protection of CA1 neurons. In contrast, only 50% of animals subjected to 2 min NT expressed hsp72 mRNA. Hsp72 induction is therefore not required for ischemic tolerance, which apparently may be achieved following insults that remain below the threshold for neuronal injury as defined by hsp72 expression.

432.4 STRESS PROTEIN INDUCTION IS NOT REQUIRED TO EXPRESS ISCHEMIC TOLERANCE IN THE GERBIL. H. Abe and T.S. Nowak, Jr.* Dept. of Neurology, Univ. of Tennessee, Memphis, TN 38163.

Income:

432.5 MAP2 IMMUNOREACTIVITY AS AN INDEX OF PATHOLOGY IN RAT HIPPOCAMPAL SLICES. Q. Zhou* and T.S. Nowak, Jr.* Departments of Anatomy & Neurobiology and Neurology, University of Tennessee, Memphis, TN 38163.

Loss of microtubule-associated protein 2 (MAP2) staining occurs in slices following anoxic incubation, and is a prominent feature of postischemic neuronal injury. Using various markers we have begun to systematically evaluate the pathology associated with slice preparation, with the goal of establishing a base line for in vitro axon/a1gycenia studies in hippocampal slices of adult rats. In this study we examined changes in MAP2 during routine slice incubation.

Male Wistar rats were decapitated under halothane anesthesia and 400 µm vibratome slices cut and incubated in artificial cerebrospinal fluid equilibrated with 95% O2:5% CO2 at 34°C. MAP2 and pre-pro-β-endorphin slices were fixed in 4% paraformaldehyde, and 50 µm vibratome sections prepared for immunocytochemistry. MAP2 was visualized with a monoclonal antibody, and pre-pro-β-endorphin with a polyclonal antibody.

The normal in vivo pattern of dendritic MAP2 immunoreactivity was found in freshly cut slices (t=0). This morphology was sometimes well-preserved through 1 h incubation, but was lost during further incubation. In many cases, however, early changes in MAP2 staining could be detected within 1 h, with preferential loss in CA1 and the upper blade of dentate gyrus, a loss that increased in thickness of the cell body, and comparable to the changes reported during anoxic incubation. Changes in MAP2 staining were well correlated with loss of Jun immunoreactivity. MAP2 therefore provides a sensitive index of slice quality that may be of general use in evaluating preparation conditions, and for comparison with other criteria of slice function.

432.6 AGING, ENERGY METABOLISM, AND THE ABILITY OF RAT HIPPOCAMPAL SLICES TO SURVIVE ANOXIA. C. Lane, C. Lonze, and J.M. Roberts, Jr.*. Department of Neurology, University of Miami School of Medicine, and *Gerrish Research Education, and Clinical Center, Miami VA Medical Center, Miami, FL 33136

We examined whether (1) decreased ATP and PCR levels before anoxia, (2) inefficient ATP or PCR use during anoxia, (3) loss recovery of ATP or PCR after anoxia, or (4) a diminished capacity for oxidative metabolism contribute to the increased vulnerability of aging brain tissue. Hippocampal slices from 6-9, 16-19, and 26-29 month old Fisher-344 rats were exposed to physiological solutions containing 5-20 mM glucose or 20 mM sodium lactate. After an initial period of normoxia (95% O2, 5% CO2), slices were subjected to anoxia (95% N2, 5% CO2). Normally was maintained by the addition of sodium lactate during anoxia with the glucose-360, 1, 2, 3, 4 and 6 h slices were fixed in 4% paraformaldehyde, and 50 µm vibratome sections prepared for immunocytochemistry. MAP2 was visualized with a monoclonal antibody, and pre-pro-β-endorphin with a polyclonal antibody.

The normal in vivo pattern of dendritic MAP2 immunoreactivity was found in freshly cut slices (t=0). This morphology was sometimes well-preserved through 1 h incubation, but was lost during further incubation. In many cases, however, early changes in MAP2 staining could be detected within 1 h, with preferential loss in CA1 and the upper blade of dentate gyrus, a loss that increased in thickness of the cell body, and comparable to the changes reported during anoxic incubation. Changes in MAP2 staining were well correlated with loss of Jun immunoreactivity. MAP2 therefore provides a sensitive index of slice quality that may be of general use in evaluating preparation conditions, and for comparison with other criteria of slice function.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
423.7RELEASE OF (3)H-D-ASPARTATE OR ENDOGENOUS GLUTAMATE FROM RAT HIPPOCAMPAL SLICES DURING IN VITRO ISCHEMIA IS MEDIATED BY A HIGH AFFINITY NA-DEPENDENT CARRIER. V. Reuterstern and J. Lipton, Dept. of Neurology, U. of Wisconsin, Madison, WI 53792.

In vitro ischemia (deprivation of oxygen and glucose/IVI) induces a several-fold increase in the release of endogeneous glutamate (GLU) and accumulated (3)H-D-aspartate (D-Asp). The D-Asp mechanism is unknown (albeit reversal of the Na-dependent high-affinity glutamate transporter has been widely suggested. We examined this hypothesis using two competitively transport inhibitors of the Na-dependent high-affinity glutamate transporter (TVA and L-trans-pyrrolidine-2-carboxylic acid (TPPC)). Slices were loaded with THA or TPPC (0.5 mM) for 60 min and then with (3)H-D-Asp (0.25 mM) for 30 min. The release was measured for 3 min intervals in 37°C during periods of IVI (10 min of ischemia). The highest release of D-Asp was induced by IVI (120 sec ischemia, 186 ± 34 pg/g dry wt) compared to control (6.7 ± 2.8 pg/g). The release of D-Asp was competitively inhibited by THA (0.5 mM) and TPPC (0.5 mM), with 55% and greater inhibition, respectively. This difference suggests that the released glutamate in vitro ischemia is largely mediated by a high affinity Na-dependent transporter sensitive to THA or TPPC but insensitive to DHIK. This phenomenon matches that of the putative neuronal carrier (UACAI = Kanai and Hedges, Nature, 360:467, 1992) and suggests that the released glutamate originates from neuronal elements. The pharmacology of inhibition of these transporter resistant suggests that glutamate releases glutamate from both neuronal and glial elements (Pines et al., Nature, 360:464, 1992).


We have previously shown that oxygen deprivation causes an increase in intracellular Ca+2 and Na+ in cortical neurons. Concomitant with these events, we have observed, in both hippocampal and cortical neurons, changes in morphology, including swelling, process retraction and death (Friedman and Hadida, J. Neurosci. (1993) 13:63, Brain Res. (1998) 615:75). We have further demonstrated that cytoskeletal elements might be cleaved during anoxia, thus making the neuron more susceptible to osmotic stress and undergoing plasticity. Cleavage of cytoskeletal elements are not involved in maintaining a cell's structural integrity, but have also been implicated in the regulation of Na+ and Ca+2 influx through, e.g. NMDA-receptors. We are focusing on actin, the major component of microfilaments, and the actin-cross-linking protein fodrin. We used cultured cortical neurons grown on poly-D-lysine coated coverslips in a chemically-defined media that does not support glut proliferation. We induced anoxia (PO2=0) on the stage of our inverted confocal microscope, confirming its effect by observing morphological changes and increased activity of Ca+2 as demonstrated by F-36. After 10 min of anoxia, the sample was rapidly removed, fixed, and, using immunocytochemistry, stained for filamentos (F-) or nonmonoclonal (G-) actin, or the 150KD cleaved subunit of fodrin. In the nonmonoclonal (G-) control, phosphatidylinositol-4,5-bisphosphate (PI(2)) staining for actin was confined to the periphery. Following anoxia, the localization of F-actin was less restricted to the periphery and the extent of staining appeared to have increased. Staining appeared for G-actin, (with DNase-I) demonstrated an apparent decrease following 10 min of anoxia. In the nonmonoclonal staining, was observed using antibodies against native fodrin, the antibody RAM-FP-150, which is specific to the cleaved subunit of fodrin did not stain. Following 10 min of anoxia, RAM-FP-150 staining could be observed, indicating that fodrin had been cleaved. These results demonstrate that cytoskeletal proteins are affected by anoxia. We speculate that early cleavage of fodrin makes the microfilaments more susceptible to morphologic disruption during swelling, resulting in an increased number of actin polymers in the cytoplasm and less well localized filamentous actin in the plasma membrane region.

423.11 ARACHIDONIC ACID PARTICIPATES IN ANOXIA-INDUCED VESICULAR GLUTAMATE RELEASE IN CA1 NEURONS OF THE RAT HIPPOCAMPUS. N. Hershkovitz* and A.N. Katchman. Georgetown Univ. Hospital, Dept. Neur., Wash., DC 20007.

Patch clamping in whole cell configuration was used to examine the effects of agents that influence arachidonate metabolism on anoxia-induced vesicular glutamate release in CA1 neuronal for 15-45 s following perfusion and ambient gas of an interface chamber from a 95%O2/5%CO2 to a 95%N2/5%CO2 mixture. As previously demonstrated, a significant increase in the frequency (340 ± 50 %), size (80 ± 5 %), and an increase in baseline miniature excitatory post synaptic currents (mEPSCs) was observed during the first 5 mins following anoxia. This increase in frequency was almost completely absent in slices which were preincubated in artificial cerebral spinal fluid containing the phospholipase A2/C/A inhibitor bromophenacylbromide (20 μM, n=8) or the cyclooxygenase inhibitors indomethacin (20 μM, n=16) and proclpin (10 μM, n=4). These observations point to a significant role for these agents on the mEPSC amplitudes. These data suggest that arachidonic acid (AA) and its cyclooxygenase products or by-products (oxygen free radicals) contribute to vesicular glutamate release during the early phase of anoxia. This observation may be important to our understanding of the neuroprotective action of these agents. (Supported by NINDS grant NS 14600-02).

423.12 HYPOXIA/HYPOGLYCEMIC-INDUCED PHOSPHOINOSITIDE HYDROLYSIS IN HIPPOCAMPAL SLICES IN VITRO: A MODEL SYSTEM FOR INVESTIGATING BIOMECHANICAL CHANGES. M. Sato, M. Matsuno, S. Watanabe, G. Bokhorst, Department of Neurosciences, Tohoku University School of Medicine, Sendai, Japan.

Cerebral ischemia induces degradation of phosphatidylinositol-4,5-bisphosphate (PIP2) and phosphatidylinositol-4-phosphate (PIP), and formation of diaiglycerol (J. Neurochem. 47, 123-132, 1986). To investigate the biochemical mechanisms involved in ischemia-induced phosphoinositol degradation, an in vitro method has been developed. Transverse slices of rat hippocampal labelled with (3)H)inositol were incubated in nitrogen-saturated and glucose-free Krebs-Henseleit bicarbonate buffer in the presence of 10 mM LiCl. Inositol phosphates and inositol phosphatidyls were analyzed by SEP-PAKs columns and TLC, respectively. The lack of glucose and oxygen induced a decrease in PIP2 and PIP. Phospha did not change. The changes were very similar to those found in vivo ischaemia. Inositol phosphates (IP) transiently increased to control levels, and inositol monophosphate gradually increased. The increase in IP5 was completely prevented by removing extracellular Ca2+ from the buffer. Addition of atropine, prazosin or ketanserin and naloxone did not affect PIP. Antagonists of HMRD and MDMA receptors also had no effect. D-jasminol and phosphoinositolphosphonate (D-AP3), a putative antagonist of metabotropic glutamate receptors, prevented the increase in IP5 and PIP. The findings of D-AP3 may be due to the inhibition of phosphatidylinositol synthesis as reported previously (Neurosci. Lett. 157, 87-90, 1993). These results provide a model system for investigating the biochemical mechanism of ischemia-induced phosphoino-
**243.15**

**SIMULTANEOUS PATCH-CLAMP RECORDINGS OF CA1 PYRAMIDAL CELLS AND INTERNEURONS IN RAT HIPPOCAMPAL SLICES: DIRECT DEMONSTRATION OF FUNCTIONAL DISCONNECTION OF INTERNEURONS FROM ANOXIA.**

E. Khazipov, P. Congar, N. Rospert* and Y. Ben-Ari

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It was proposed in our previous work (Khazipov et al., *Neurophysiol., 70 : 229I-2260, 1993) that high sensitivity of polysynaptic systems to anoxia is due to the functional disconnection of inhibitory interneurons from excitatory inputs. To verify this hypothesis, the effects of anoxia were studied in simultaneous whole-cell patch-clamp recordings from interneurons and pyramidal neurons in CA1 region of adult rats hippocampal slices; these were both identified by electrotonic properties and by their sensitivity to bicuculline. Anoxia (95%N2-5%CO2; 5 min) generated currents in interneurons similar to pyramidal cells: the most prominent were the anoxic outward current and the postanoxic outward current. EPSCs and polysynaptic IPSCs were depressed by anoxia, and this depression was more pronounced in the interneurons. The currents evoked by pressure ejection of glutamate, AMPA and NMDA were not affected by anoxia, suggesting that the depression of EPSCs in both interneurons and pyramidal cells is due to a presynaptic mechanism. Depression of EPSCs but not polysynaptic IPSCs nor GABA receptors mediated component of monosynaptic IPSCs (see Congar et al. *Soc. Neurosci. Abst., 1994) was prevented by adenosine A1 receptor antagonists DPCPX (200mM) and DPCPX (200mM) indicating the involvement of presynaptic adenosine receptors in anoxic depression of EPSCs. These observations indicate that anoxia preferentially depresses excitatory inputs to anoxia leading to a functional disconnection of interneurons.

**243.16**

**DIFFERENTIAL RESPONSES OF CA1 PYRAMIDAL CELLS AND GRANULE CELLS IN THE DENTATE GYRUS TO ISCHEMIA-LIKE CONDITIONS IN RAT HIPPOCAMPAL SLICES.**

H Shintani, Y. Fujito*, A. Mizuno and M. Adki

Dept. Physiol., School of Med., Sapporo Med. Univ., Sapporo 060, Japan

We examined the regional difference of responses to oxygen-glucose deprivation (ischemia-like conditions). In cultured rat hippocampal slices, we measured the effects of oxygen-glucose deprivation on the hippocampal and dentate gyrus of DG in rats hippocampal slices (350mm thick). Changes of resting membrane potential (resting Vm) and antidiomatic action potentials (AAPAs) were compared between CA1 pyramidal cells (PCs) and granule cells (GCs) in DG. CA1-P (measured by Fura-2/AM loaded slices were also compared between CA1 and DG. In PCs, Hypo-GC(1) produced an initial hyperpolarization and a subsequent rapid depolarization. By contrast, GCS showed a slight initial hyperpolarization and a subsequent stowel depolarization than PCs. In PCs and GCS, AAPAs disappeared after 7 to 9 min periods of Hypo-GC(1), when rest Vm depolarized to Hypo-GC(1). In contrast, DG was not affected by Hypo-GC(1), and did not produce an irreversible depolarization in both neurons. However, when reoxygenation was started at the time of disappearance of AAPAs, reoxygenation occurred in 86% (12/14) of PCs and 36% (5/14) of PCs. (CA2+) remarkably increased at the depolarization phase of both DG and PC. The peak of [Ca2+] were significantly higher in CA1. Under CA2+-free medium, peak [Ca2+] decreased and there was no significant difference between CA1 pyramidal cell layer and granule cell layer. These results demonstrate the differential responses to Hypo-GC(1) between CA1 PCs and GCs in DG, probably due to different distributions of ion channels of these neurons.
BRIEF PERIODS OF OXYGEN AND GLUCOSE DEPRIVATION IN VITRO CAUSE SWELLING AND DELAYED CELL DEATH. W.J. Goldberg*, S. Stroke & Trauma Lab., Dept. Veterans Affairs Medical Center, Washington, DC 20422.

An in vitro system was used to mimic several aspects of ischemia, including cell swelling, delayed activity, glycolysis, deoxygenation, and prolonged reperfusion. We replaced normal culture medium from 3-week-old spinal cord cultures with oxygen-depleted, chemically balanced (310 mM) medium (25 mM Na, 65 mM K and 1 mM Ca). After incubation at 37°C for 15 minutes in an atmosphere of 7% CO2/93% N2, the cultures were returned to normal, oxygenated culture medium for 0.5 or 96 hours. At the end of each recovery period, cultures were immunohistochemically stained using combined antibodies to 68K, 150K, and 200 K neurofilament proteins. In non-deprived cultures, the neuronal density was 100 cells/100 mm². These cells were 96.8±6.8 µm (means±SEM) in size. More than 89% of the original neuronal population survived the 15 minute deprivation period. Two classes of cells were observed based on cell size: one group was not different from non-ischemic neurons (109±7.6 µm) and the other was significantly swelled (255.3±25.5 µm, p<0.05). The survival rate decreased to 64.7% after 1 hour of recovery. Swollen (240±14.3 µm, p<0.05) and non-swollen (112±9.3 µm) neuronal populations were observed at this time. After 4 days of recovery, the survival rate decreased further to 23%. All deprived cells were swollen to 337±47.7 µm (p<0.05). No normal sized neurons were observed. Supported by the Department of Veterans Affairs.

ISCHEMIA: MECHANISMS II

424.1 SUBCELLULAR LOCALIZATION OF ISCHEMIA-INDUCED INHIBITION OF CaMKII IN THE HIPPOCYMAL BRAIN SLICES: SIMULTANEOUS Ca2+ AND KCN POTENTIAL MEASUREMENTS. M.J. Evans* and C.D. Estabrook, Biophysics Dept., SmithKline Beecham, Harlow, Essex CM19 5AD U.K.

To investigate synaptic responses, ischemia depolarisation, and Ca2+ influx under oxygen and glucose free conditions, 400 micron hippocampal slices were loaded with the Ca2+ indicator dye Indo-1 AM. Microspectrophotometry was used to monitor changes in fluorescence intensity at a 20 µm diameter window centred over the stratum pyramidale of either CA1 or CA3. Extracellular DC recordings were made in the stratum pyramidale adjacent to the window. Experimental brain temperature was 36°C. The relevant artefactual pathway was stimulated every 15s to monitor synaptic responses. Synaptic responses in Indo-1 loaded slices were similar to those of control slices. On switching from normal medium to 2% saturated, glucose-free medium, synaptic responses were eliminated within 90s. After 276±45s, a large, transient, negative DC shift was observed in CA1 which was closely followed by a rapid and sustained rise in [Ca2+]. Reperfusion with normal medium shortly after the peak [Ca2+] resulted in a return of [Ca2+] to baseline level over 3 to 5 min, but there was no recovery of the synaptic responses. Switching to normal medium before the depolarising shift and [Ca2+] rise resulted in a slow recovery of synaptic transmission to near baseline levels. Minoxidil containing only CA1 gave similar responses, indicating that the CA1 events are not driven by preceding events in CA3. The depolarising wave and [Ca2+] rise in CA3 had a longer latency (59±57s) and rose more slowly to a lower peak amplitude. Repetition of the stimulus after the peak [Ca2+] rise in CA3 resulted in a sustained recovery of synaptic responses. These regional differences in response to acute oxygen and glucose free insult of CA1 to injury relative to CA3 observed with in vivo ischaemia models.

424.2 TRANSLATION OF Ca2+-CALMODULIN PROTEIN KINASE II IN THE VULNERABLE NEURONS FOLLOWING ISCHEMIA AND HYPOGLYCEMIA IN THE RAT. Bing-Yi Huang, Tetsuo Kurihara, Fredrik Kamme and Tadeusz Wieloch, Lab. for Exp. Brain Res., University Hospital, 221 85 Lund, Sweden.

Alterations of Ca2+-calmodulin-dependent protein kinase II (CaMKII) during and following transient cerebral ischemia (TICI) and insulin-induced hypoglycemia (HG) in the rat were studied. CaMKII was translated to synaptic junctions after both TICI and HG as showed by immunohistochemistry and calmodulin binding, concomitant with a decrease in CaMKII activity. The CaMKII activity increases transiently and lasts longer than HG, which is consistent with the CaMKII-dependent translocation to synaptic junctions. The ischemia also reduces the expression of α-subunit of CaMKII mRNA in dentate gyrus as demonstrated by in situ hybridisation. The results suggest that a stimulation of CaMKII is ongoing in the vulnerable areas which causes translation. These results suggest that the role of CaMKII signal after ischemia. Intrinsic ischemia inhibits protein neuronal damage as well as reduces the translocation and increases mRNA expression of CaMK-II induced by ischemia. In conclusion the persistent translation is highly correlated to neuronal damage in both ischemia and hypoglycemia.

424.3 ALTERATIONS IN Ca2+/CaMK-Kinase II AND OTHER PROTEINS IN POSTSYNAPTIC DENSITIES AFTER FOREBRAIN ISCHEMIA IN RATS. Jalajee Ammala*, James C. Glose, Department of Neurology, University of Texas Health Science Center, Houston, Texas 77025.

Postsynaptic densities (PSDs) located beneath the postsynaptic membrane may be the organizer for organizing channels, receptors and enzymes that participate in signal transduction. The major PSD protein, α-subunit of Ca2+/calmodulin-dependent protein kinase II (CaMK-II), accounts for up to 30-40% of total PSD proteins. Several studies have shown that CaMK-II expression occurs after ischemia and hyperglycemia, translation of CaMK-II from cytosol to post-synaptic fraction, and that these changes are reversible after brief but not prolonged duration of ischemia. The present study analyzes the expression of CaMK-II in tissue sections from rats subjected to ischemia. Winter rats were subjected to 20 min of ischemia using a four-vessel occlusion (4VO) model. After 0, 24 and 48 h of reperfusion, animals were decapitated and forebrain isolated. Tissue was processed for immunohistochemistry and results were compared with control groups. Immunohistochemical analysis revealed that the expression of CaMK-II was increased in both the basal ganglia and cerebral cortex. The results of this study indicate that CaMK-II is expressed in both biologically relevant tissues in ischemic and non-ischemic conditions. Further research is needed to determine the role of CaMK-II in the recovery from ischemia.

424.4 A reversible elevation of free calcium during simulated ischemia in neuronal tissue culture. J.J. Varon*, M. Han, S.C. Jo and A.G. Thomas, Dept. of Neurology and Neuroscience Johns Hopkins School of Medicine, Baltimore, MD 21205.

Previously, we have described tissue culture models of ischemia using metabolic inhibitors and reversible ATP depletion. Neurons can be protected by NMDA receptor blockade after simulated ischemia, allowing delayed treatment of injury. Since the accessibility of cultures is preserved, we have been able to measure free calcium changes during simulated ischemia with the sensitive dyes fluo-3 or fura-2 in dissociated cortical cultures. Intracellular calcium rose reversibly during metabolic inhibition. The calcium rise was slower and the recovery faster than that caused by bath application of glutamate. Blockade of NMDA receptors with MK-801 caused no change in the calcium increase, but removal of extracellular calcium decreased the rise.

Calcium entry during recovery was investigated histochemically. Calcium permeable non-NMDA receptor channels are permeable to cobalt. Cultures were perfused with 100 µM cobalt visualized with ammonium sulfide precipitation followed by silver intensification. In both dissociated cortical cultures and organotypic hippocampal cultures, some neurons accumulated extracellular calcium during recovery from simulated ischemia. More cells were stained during bath application of kainite or glutamate. These experiments suggest that while free calcium may rise during simulated ischemia, toxic, persistent calcium entry during recovery may not be reflected by increased intracellular calcium.
424.5 EFFECTS OF TRANSIENT CEREBRAL ISCHEMIA ON CALCIUM-BINDING PROTEIN mRNA LEVELS IN THE GERBIL HIPPOCAMPUS. A.M. Babcock1, J. Noltkamper, C.M. Padin2, P.E. Miccdyld2 and P. Popper. Dept. of Psychology and Biology, Montana State Univ., Bozeman MT 59717, Dept. of Anat. & Cell Biology, UCLA, School of Med., Los Angeles, CA 90024. Previous studies have reported that hippocampal calcium calmodulin kinase II (CaM II) mRNA levels decrease at 24 hrs following transient ischemic insult in the gerbil (Hinestrosa & Kindy, 1992). The present study attempted to confirm and extend these findings by evaluating changes in hippocampal CaM II mRNA levels using in situ hybridization. Gerbils (n=5/group) underwent a 2-min bilateral carotid occlusion or sham procedure. At 2 hrs post surgery, locomotor activity was assessed as a behavioral marker of ischemic cell damage. Gerbils were sacrificed at 24 hrs and sections containing the hippocampus were processed for in situ hybridization with cRNA probes for the α and β subunits of CaM II (separate sections). As expected, ischemic gerbils exhibited higher activity levels indicative of hippocampal damage. Changes in enzyme activity following ischemia were assessed using HPLC with an electrochemical detector (EMI) and sodium nitroprusside inhibition. These results are discussed in relation to observed alterations in CaM immunoreactivity. Supported by American Heart Grant-in-Aid, P20 RR01927-01 (AMB) and NIH-NINCDS (PEM).

424.6 SUSTAINED INHIBITION OF EXCITATORY SYNAPTIC TRANSMISSION BY ADENOSINE DURING MODERATE HYPOXIA. Alice L. Fournier, Michael S. Kaplan, and K.S. Leu. Dept. of Neurological Surgery, Univ. of Virginia, Charlottesville, VA 22908. Adenosine is a potent inhibitor of excitatory synaptic transmission at many sites in the CNS. During ischemia and hypoxia, extracellular adenosine levels increase substantially. This elevation of adenosine appears to contribute to the initial phase of the hypoxic inhibition of synaptic transmission (Curtis et al., Brain Res. 400, 37-42, 1986). The present study examined whether adenosine contributes to later phases of hypoxic inhibition of synaptic responses during 1 hour of moderate hypoxia. Hippocampal slices prepared from adult Sprague-Dawley rats were submerged in medium with an oxygen tension of approximately 470 mm Hg. Field excitatory postsynaptic potentials (EPSPs) were recorded in the stratum radiatum of CA1 in response to stimulation at the CA1-CA2 border. Hypoxic conditions were established by changing the oxygen tension in the superfusion medium to 85 mm Hg. A complete inhibition of EPSPs was observed within approximately 9 minutes of hypoxia. An injection of adenosine 1A receptor, cyclopentyltheophylline (CPT; 10μM) was added to the medium at various intervals after the onset of hypoxia. The addition of CPT to the hypoxic superfusion medium after the loss of synaptic responses resulted in a substantial recovery of EPSPs, while the loss of synaptic responses remained complete for the entire hour of hypoxia in the absence of CPT. When CPT was added 10 or 20 minutes after the loss of synaptic responses, EPSPs recovered to 68% and 47% of their prehypoxic baseline values respectively. These results indicate that adenosine-mediated inhibition contributes significantly to the sustained loss of EPSPs during hypoxia. These findings suggest that adenosine functions to maintain excitatory synaptic transmission at low levels during prolonged moderate hypoxia and support the concept that adenosine serves as an endogenous neuroprotectant.
ISCHEMIA: MECHANISMS

424.11 CHANGES OF ACETYLCHOLINE IN RAT BRAIN FOLLOWING ISCHEMIA. H. Kanemitsu1, K. Kawai1, T. Kirino2, A. Tamura3, K. Iwasaki3, M. Fujitani3, 1Department of Neurosurgery, Teikyo University School of Medicine, Tokyo, Japan. 2University of Tokyo. 3University of Fukuoka.

We have reported that the decrement of the acetylcholine level may be related to the disturbance of spatial cognition. The purpose of this present study is to determine change of the acetylcholine level in ischemic and normal brain tissue following focal ischemia. Male Wistar rats weighing 280-300 g were anesthetized with 2% halothane inhalation and the proximal portion of the left middle cerebral artery (MCA) was occluded by the microsurgical technique. The animals were treated with microwave 1.2 and 2.4 weeks following MCA occlusion and the acetylcholine levels of the cerebral cortex, caudate putamen and thalamus were measured by using HPLC-ED system. The acetylcholine levels of the cerebral cortex and caudate putamen on the ischemic side in the MCA-occluded group were significantly decreased compared with those in sham-operated group. However, the levels of the opposite side and thalamus unchanged. Those decrements of acetylcholine may be caused by interception of the neurons in the Meynert nucleus and be related to the impairment of learning behavior.

ISCHEMIA: APOTOTIS


The CA1 pyramidal neurons in the hippocampal area are selectively vulnerable to transient anoxic-ischemic damage. In experimental animals the CA1 pyramidal neurons undergo cell death several days after brief forebrain ischemia. It remains, however, unknown whether this delayed neuronal death is necrotic or apoptotic. We examined the degenerating process of the CA1 pyramidal neurons in gerbil hippocampus after brief ischemia. Neurites of the CA1 neurons were nick end labeled by biotinylated dUTP mediated by terminal deoxy- nucleotidyl transferase 3 and 4 days after ischemic insult, but not at the prior stages. Simultaneously, dense chromatin masses appeared in nuclei of the neurons. By electrophoretic analysis, laddering of DNA occurred only in CA1 hippocampal tissues obtained 4 days after ischemic insult. The fragmented DNA in the CA1 pyramidal layer was phagocytosed by microglial cells. The results suggest that delayed death of the CA1 pyramidal neurons after brief ischemia is not necrotic but apoptotic.


Brain ischemia can result in oxidative stress leading to the formation of free radicals. These free radicals can potentially exert cytotoxic actions by causing chemical modifications to lipids, proteins, and DNA. Damage to critical parts of the DNA molecule could lead to cellular dysfunction and further tissue injury. In the present study, we have begun to examine the sensitivity of normal brain cells to DNA damaging agents and the ability of those cells to repair damage. Neurons, astrocytes, and cerebral endothelial cells were isolated at day 15 of gestation, at postnatal day 1, and postnatal day 14, respectively, planed into plastic culture dishes, and grown to confluence. Those cells were exposed to radiation doses of 6, 8, 10, and 32 Gy to induce DNA double-strand breaks (DSBs) and then incubated for 0 min to 48 hrs to allow repair of the DNA damage. The number of DSBs remaining at the various time points was determined by subjecting the DNA to pulsed-field gel electrophoresis. There was a linear increase in the number of DSBs with radiation dose. There were two components to DSB repair: a fast component with half-times of repair of 10-15 min and a slower component with half-times of repair of 3-7 hrs. While there was no difference between astrocytes and cerebral endothelial cells in the rate of repair, the rate of repair increased by 10% relative to the other cell types. Treatment of astrocytes with 0.25 mM dibutyryl cyclic AMP decreased the number of DSBs induced and significantly increased their rate of repair. This results indicate that cyclic AMP can enhance repair of DNA damage and that neurons are better able to repair DNA damage than other brain cell types. Supported by NIH Grants AG 08938, NS 14543, NS 25372, CA 13522, and the American Brain Tumor Association.

425.4 DETECTION OF DNA STRAND BREAKS IN RAT CORTICAL NEURONS EXPOSED TO FOCAL ISCHEMIA-REFURFUSION IN VIVO. C. Do, R. Hu, S. Wong, D.W. Choi, C.Y. Hsu*. Dept. of Neurology and Center for the Study of Nervous System Injury, Washington University School of Medicine, St. Louis, MO 63110.

In our rat model of focal middle cerebral artery ischemia-refurbation, coagulation necrosis occurs confined to the distribution of the artery. In the infarcted tissue, neuronal death evolves over several days after the ischemic insult. Some recent studies have suggested that neuronal DNA fragmentation may accompany focal ischemic injury, raising the issue of apoptosis. The present study was conducted to look for evidence of DNA strand breaks in our ischemia model. Genomic DNA isolated from the ischemic cortex was subjected to non-denaturing polyacrylamide gel electrophoresis. Twenty-four and 48 hr after ischemia, DNA fragments of various sizes were seen. The O-HH DNA ends were immunohistochemically identified in the formalin-fixed, paraffin-embedded brain slices by in situ end labeling (using terminal transferase with dUTP-digoxigenin) followed by anti-digoxigenin-peroxidase immunohistochemistry. DNA strand breaks detected by such immunostaining was not observed in non-ischemic cortex, or in controls stained without dUTP-digoxigenin, anti-digoxigenin antibody, or terminal transferase. Strong immunoreactivity was noted in the nuclear regions of injured and near normal neurons. The detection of DNA strand breaks at 2 different methods supports the idea that cortical neuronal death in focal ischemia may involve endonucleolytic degradation of DNA similar to that noted in several forms of apoptosis.
ISCHEMIA: APOPTOSIS

WEDNESDAY AM

425.5
DNA FRAGMENTATION IS NOT AN EARLY EVENT IN ISCHEMIC NEURONAL DEATH. P. Wu* and J.M. Davis. Dept. of Neurology, SUNY at Stony Brook, Stony Brook, NY 11794-8121.

DNA fragmentation is thought to be an early, perhaps even, initial event in programmed cell death. We have been exploring the hypothesis that ischemic cell death may be a form of programmed cell death in focal and transient global ischemia in gerbils. After 5 minutes of bilateral carotid artery occlusion, hippocampal CA1 neurons undergo a delayed cell death. We studied DNA using 2% agarose gels at various times after transient carotid occlusion from whole hippocampus (3 experiments) and hippocampal CA1 region (1 experiment), and punches of CA1, pyramidal cells (3 experiments). DNA from dexamethasone-treated whole hippocampus in the gerbil was fragmented at 24 hours after carotid occlusion. However, we did not see DNA "laddering" either at late times (48, 54, 66, 72 hours) or at earlier times (2, 4, 6, 24 hours). While this was in progress, El Khoury et al (1993) reported "laddering", from gerbil whole hippocampus between 54 and 96 hours after carotid occlusion. We found no evidence to reproduce their results even using their extraction technique, but were unable to confirm their findings.

We found DNA fragmentation in focal ischemia as reported by others. After permanent ligation of one carotid artery, half the gerbils sustained an infarct (as assessed by TTC staining); some also had seizures. "Seizures" were present at 24 hours and more obvious at 48 hours, but were not present at 3 or 6 hours after ligation. In focal ischemia, DNA fragmentation was a late phenomenon appearing after necrotic cell death in the infarcted area.

We conclude that DNA fragmentation does not trigger ischemic cell death as has been proposed. Further, it appears that DNA "stepladders" can be seen as a late event in necrotic cell death. [Supported by the NIH (NS 05599) and the VA]

425.7

Previous work provided evidence for an apoptotic component in the cell death in rat brain following global ischemia produced by two-vehicle occlusion (2VO) (Maxman et al., Neurosci. Letts. 164, 89-92 (1993)). Further studies undertaken to establish a time course for the development of this damage following 12 min 2VO showed an increase in damage with increasing reperfusion time. Cell death was quantified by two methods. Firstly, DNA was radiolabeled with diodeoxyATP at 3 h OH and the labeled DNA below 10K following gel electrophoresis was measured. Secondly, 3' ends were labeled in situ on fixed sections taken from ischemic brain to identify those cells containing fragmented DNA. A comparison of these two parameters was undertaken for the striatum. This gave a positive correlation, thereby supporting the idea that the internucleosomal cleaved DNA seen by gel electrophoresis is derived from the cells identified by in situ labelling and that this provides a quantitative measurement of the cell death. It was also apparent that fragmented DNA could be detected at the same time that morphological changes in the nucleus were observed.

To further confirm an apoptotic component in the death of cells following transient ischemia a competition was made with the necrotic cell death produced by decapitation. Degraded DNA was detected as a smear after electrophoresis, not as discrete bands. A time course study showed that selectivity in the hippocampus differed from that seen following ischemia with fragmented DNA first being detected in the dentate gyrus, followed by CA1, then CA3.

Further support for an apoptotic process being activated following ischemia is provided by these results. The time course for ischemia damage determined by conventional staining techniques correlated with that for DNA cleavage and major distinctions were observed between ischemic cell death and necrotic cell death.

425.8
APOPTOSIS AND NECROSIS CONTRIBUTE TO DELAYED CELL DEATH FOLLOWING HYPOXIC-ISCHEMIC INJURY TO THE IMMATURE RAT BRAIN. E.J. Beilharz, C.M. Williams, M. Dragunow *, E.S. Striimane, R. French *, P.D. Gluckman*. Children's Hospital and Centre for Developmental Medicine and Biology, Department of Pharmacology, Dept. of Anatomy, University of Auckland, Auckland, New Zealand.

The mechanisms leading to delayed cell death after hypoxia-ischemia in the 21 day old Wistar rat brain following either mild (15 min) or severe (60 min) unilateral hypoxic-ischemic injury. The timecourse of DNA degradation (using gel electrophoresis and in-situ end labelling), microglial activation (using immunoelectron microscopy for Iba1 staining) and cell death (TACS1 staining) and c-jun expression were examined. The mild injury led to selective neuronal loss. This was associated with the development of apoptotic morphology, DNA laddering and acidophilia from 3d post-hypoxia. Prolonged expression of c-jun occurred in these neurons before death. Cell death was accelerated by the severe injury, with DNA degradation and apoptotic morphology seen at 10h in some regions. However, in the infarcted cortex a different pattern was seen: the DNA and microglial remained intact, and cells basophilic, until after 10h post-hypoxia, then widespread necrosis developed by 24h. In contrast to regions of selective neuronal loss, DNA degradation was initially random (at 24h), with laddering not detected until 3d. Microglial activation coincided with the onset of DNA degradation in regions of selective neuronal loss (in both models) but not infarction. The results clearly indicate that there are two distinct pathways to cell death: cortical infarction was necrotic, and occurred independently of microglial activation and apoptosis. In contrast, selective neuronal death was apoptotic and closely coupled to the microglial reaction and c-jun expression.

NEUROTOXICITY: RAA 1A

426.1

Methamphetamine (METH) damages nigrostriatal DA nerve terminals in rodents and monkey brain. Pretreatment with alpha-methyl-p-tyrosine or DA receptor antagonists protect against the toxic effects of METH, suggesting that at least one component of the neurotoxicity involves the ability of METH to increase excitotoxic concentrations of DA. NMDA antagonists are also capable of blocking the neuronal-damaging effect of METH, suggesting that alterations in glutamatergic neurotransmission may be involved as well. However, several of the NMDA antagonists employed (e.g., MK801, PCP) are also reported to block excitatory amino acid (EAA) receptors, raising the possibility that a reduction in DA neurotransmission, rather than NMDA receptor blockade, underlies their neuroprotective actions. We therefore determined the abilities of two NMDA antagonists, MK801 and PCP, to reduce METH-induced DA release in the rat striatum using in vivo microdialysis, and also determined tissue levels of striatal DA in these same animals one week after drug injection. The administration of METH (10 mg/kg i.p.) each 2 hr on 4 consecutive days caused large increases in striatal ECF DA (> 500%). Pretreatment with MK801 (2 mg/kg i.p.) each 2 hr for 4 days caused no significant changes in striatal ECF DA (compare: 13.3 ± 2.2 nM in control) and METH-induced DA overflow. In a similar fashion, pretreatment with CGS19755 (35 mg/kg i.p.) each 2 hr on 4 days, 4 min before METH, i.p.) also failed to significantly alter the METH-induced DA overflow.

426.2
METHAMPHETAMINE-INDUCED DEPLETION OF GLUTAMATE-POSITIVE NEURONS IN THE SOMATOSENSORY CORTEX OF THE RAT. C. Pu and C. Y. Vorhees*. Children's Hospital Research Foundation, University of Cincinnati, Cincinnati, OH 45229, USA.

The neurotoxic effects of methamphetamine on dopaminergic and serotonergic terminals have been well documented. However, the neurochemical characteristics of degenerating neurons in the somatosensory cortex are unknown. By using immunocytochemical techniques for glutamate immunohistochemistry, it was found that MA exposure in adult rats (10 mg/kg given 4 times i.p at 2 hour intervals) causes localized depletion of glutamate-positive neurons three days following treatment. The affected region is in the dorsal sensory cortex, central to the somatosensory cortex. first-third portion of the longitudinal fissure to the rhinal sulcus. The depletion of glutamate-positive neurons occurred in all layers of the cortex with layers II-III showing the most severe loss. The survival of neurons in the affected regions is consistent with that demonstrated previously with silver staining following methamphetamine, amphetamine and methyldiosoxymethamphetamine (MDMA) exposures. The results indicate that MA exposure results in the degeneration of glutamate neurons in the somatosensory cortex of adult rats.

Activation following a single kindling stimulus to the amygdala. We have been examining the role of c-fos in the development of amygdala kindling using antisense technology. We have performed studies, animals received infusions into the amygdala via a chronically implanted cannula either once (Group 1) or on a daily basis (Group 2) with phosphorothioate antisense (AS) or control (C) oligonucleotides. Ten hours after the infusion rats in both groups were administered a kindling stimulus in the amygdala and an electroencephalographic afterdischarge (AD) was recorded. The AS treated animals in Group 1 showed considerable attenuation of Fos-immunoreactivity when compared to the C treated animals. However, during the course of these studies we observed that subsequent to 3-4 infusions Group 2 animals (both AS and C) no longer demonstrated ADs. Upon histological examination of these animals, lesions to the amygdala and surrounding areas were observed. Reactive gliosis was demonstrated surrounding the lesion with glial fibrillary acidic protein immunoreactivity (GFAP-IR). These detrimental effects are dose related. Further studies have now revealed that the degree of histological damage can be greatly minimized by extending the period between infusions. These results suggest that certain cautions must be taken when using antisense technology and furthermore that only certain types of experimental approaches are amenable to it.

Supported by MRC Canada and the Savoy Foundation.

MODULATION OF EXCITOTOXIC DAMAGE BY INTERLEUKIN-1 (IL-1) AND IL-1 RECEPTOR ANTAGONIST (IL-1ra).

Lawrence C. Prather, Neuroscience Division, 124, School of Biological Sciences, University of Manchester M13 9PT, UK.

We have previously suggested that the cytokine IL-1 is involved in ischemia and excitotoxic brain damage, since recombinant human IL-1ra (rhIL-1ra, Synergen, USA) inhibits neurodegeneration caused by focal ischemia or NMDA receptor activation in the brain. Neurodegeneration has been ascribed to overactivation of AMPA as well as NMDA receptors. In the present study we have therefore compared effects of rhIL-1ra and recombinant human IL-1ra (rhIL-1ra) on damage induced by infusion of an NMDA (cis-2,4-methanoglutamate, MGlu) or AMPA (S-AMPA) receptor agonist into the striatum of the rat brain. Both agonists induced reproducible lesions assessed histologically. In both studies, animals receiving rhIL-1ra (5ug) with the excitatory amino acid agonist (100mM MGlu or 150nm S-AMPA) significantly inhibited damage (lesion volume) by 46% or 43% when induced by NMDA or AMPA receptor activation respectively. In contrast, infusion of IL-1β (14pg, 100U) did not affect either form of damage (the dose of exogenous used was 5nmol MGlu and 10nmol S-AMPA). Additionally IL-1β did not induce neurodegeneration when infused alone. These data indicate that endogenous, but not exogenous IL-1, mediates NMDA and AMPA receptor induced neurodegeneration.

CIS-FLUPHENIXOL PROTECTS STRIATAL CELLS FROM MALONATE-INDUCED LESIONS.

P. Hantrave*, M.C. Guiraud, S. Paillot and B. Bruguier. CNRS URA 1385, Service Frédéric Joliot, D1PP-DSV, 94100 Orsay Cedex and INSERM CIP 91-02, Hôpital Henri Mondor, Créteil 94010, FRANCE.

Malonate, a succinate dehydrogenase inhibitor, has been shown to produce striatal lesions resembling Huntington’s disease striatal degeneration. Treatment of adult rats with malonate (1 mmol/kg body weight, i.p.) 1 hr after birth (n = 10) produced a significant reduction (P < 0.05) of striatal dopamine levels (92%) and protein content (78%) compared to controls. On Day 21, the rats were injected with 2% Fluorochrome Blue and Nissl stains were observed. The malonate-treated rats showed a significant reduction in the number of Nissl-stained neurons (28% vs. controls). In contrast, the rats treated with 2% Fluorochrome Blue had a significant increase in the number of Nissl-stained neurons (132% vs. controls).

CIS-FLUPHENIXOL has been shown to be beneficial in Huntington’s disease patients. The mechanism of action of this drug is not well understood. However, it is believed to be mediated by a glutamatergic system which plays an important role in the pathogenesis of this disease. The current study was therefore designed to evaluate the protective effects of CIS-FLUPHENIXOL on malonate-induced striatal lesions in the neonatal rat.

METHOD: Adult rats, injected intraperitoneally (pretreatment at 30 min and post-treatment 60 min after) with or without saline (controls) were injected with a single malonate injection (1 ml/kg) into the left striatum. At one week survival, the CIS-FLUPHENIXOL group showed a significant improvement in striatal survival. Compared to controls, CIS-FLUPHENIXOL provided more than 50% protection against malonate-induced striatal lesions.

DIFFERENT RESPONSES TO N-METHYL-D-ASPARTATE AND KAINIC ACID IN CEREBELLAR GRANULAR CELLS OF LEAD-EXPOSED PUPS.

D.K. Lim*, T. Boa and I.K. Ho*.

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To determine changes in response to N-methyl-D-aspartate (NMDA) and kainic acid (KA) in cerebellar granular cell of offspring lead mothers, pregnant rats received 0.25% lead acetate in the drinking water 2 days after gestation. The control group was given sodium acetate (0.125%) in drinking water. The cerebellar granular cells were cultured from 7 to 8 day old pups. Changes in the level of cytochrome c* (CIT) and glutamic acid release of glutamic acid were measured using triphenyltetrazolium chloride (TTC) and using fluorometric assay. In the presence of cytochrome c*, the maximum responses to KA were not different. The NMDA-induced elevation of CGMP was not affected in the lead exposed pups. However, the degree of KA-induced increase in CGMP level in cerebellar granular cells of lead exposed pups was significantly reduced. The increase in CGMP level in control pups was 55.5 and 51.4% of the control groups when the incubations were carried out for 3 min and one hour, respectively. The NMDA- and KA-stimulated release of glutamic acid in cerebellar granular cells prepared from lead exposed pups was also significantly reduced. These results indicate the lead exposure to the mother affect the excitatory amino acid systems during the development of the offspring.

The effects of a mitochondrial toxin 3-NPA on operant performance, dopamine (DA), serotonin and their metabolite concentrations in selected brain regions were studied in adult male Sprague-Dawley. Rats were challenged by caesarean section as either noninsulted (NI) or insulted (I) with hypoxia. Hypoxia was produced by submerging dissected uterine horns in saline for 15 min. NI rats were siblings of the I rats delivered from the saline-exposed pregnant animals. Rats were cross-fostered and behavior was assessed. At 1 year of age, NI and I rats were injected with 3-NPA at 5 mg/kg/day (Monday-Friday). The dose was increased by 5 mg/wk reaching 30 mg/kg/day. After sacrifice at the end of the NPA treatment, brains were dissected, weighed and analyzed for neurotransmitters. Operant measures significantly decreased with increasing 3-NPA doses but were not differentially affected by hypoxia. Ataxia at the highest dose of 3-NPA was observed only in NI rats (3/9 rats). A trend toward a DA concentration increase in frontal cortex was seen in both I and NI groups. While weights of brain regions in the control and 3-NPA treated I rats were similar, cerebellar weight in NI rats after 3-NPA treatment was reduced (ANOVA, p < 0.001). Therefore, perinatal hypoxia insult may decrease subsequent sensitivity to the toxic effect of 3-NPA.


Systemically administered L-chloropropionic acid (CPA) produces selective damage to cerebellar granule cells. We investigated whether CPA may produce the granule cell damage by acting at a subtype of the N-methyl-D-aspartate (NMDA) receptor. Groups of rats were treated with CPA (10 mg/kg/po). Some animals received the irreversible NMDA antagonist, MK-801 alone (5mg/kg/po), saline or CPA plus MK-801. The neurotoxicity was assessed at days 3 and 5 of the study. An increase in locomotor activity was observed. CPA alone showed marked locomotor retardation which became very severe by days 3 and 4. These animals showed marked weight loss (-22% of controls) and were terminated at days 3 or 4. In the CPA plus MK-801 treated animals although showing a small weight loss early on in the study they did not show abnormal locomotor activity by days 2 to 5. Neuropathological examination of the brains from CPA-treated rats showed a marked (80-90%) loss in cerebellar granule cells by day 3 and 4 of the study. The co-administration of MK-801 with CPA was able to completely prevent damage to the cerebellum. MK-801 was also able to prevent the CPA-induced loss of NMDA receptors in the granular layer of the cerebellar cortex and concentrations of aspartate and glutamate in cerebellum. Finally there was a complete prevention of the CPA-induced cerebellar edema by MK-801. In conclusion we suggest that CPA is toxic to rat cerebellum because this brain region contains a subtype of NMDA receptor with unique pharmacology and regulation.

ANTI-PHENCYCLINE MONOClonAL FAB FRAGMENTS DRAMATICALLY DECREASE PHENCYCLINE (PCP) NEUROTOXICITY IN SPRAGUE-DAWLEY (SD) RATS. J.L. Valentine, W.D. Westerling and S.M. Olson* Dep't of Pharmacology & Toxicology, Univ. of Ark. for Med. Sci., Little Rock, AR 72205.

High affinity Ki=1.8 nM anti-PCP monoclonal Fab fragments were used to reverse in vivo PCP-induced behavioral toxicity. For these studies, male SD rats (n=4 per group) were administered four treatments in random crossover design. 1) An i.v. dose of 1.0 mg/kg saline vehicle, followed 5 min later by 1.0 ml saline (Saline-saline control). 2) All others received a previously active dose of PCP (1.0 mg/kg) followed 5 min later (as toxicity began to maximize) with 3) saline (PCP-saline, protective control). 3) anti-PCP anti-Fab (PCP-anti-PCP Fab) at 0.3, 1.0 and 3.0 times the 1.0 mg/kg PCP dose, and 4) nonspecific Fab (non-specific Fab) at the same doses as the anti-PCP Fab. To assess PCP and treatment effects, locomotor activity was determined over 2-min intervals for a 60-min period on an open field apparatus. Changes in 2D activity monitoring were monitored for 10 min prior to saline or PCP administration. Saline-saline injections resulted in relatively low background counts (25 counts per 2-min interval). PCP-saline injections increased the baseline levels of locomotor activity, with an initial response of approximately 150 counts 10 min and a duration of 39 ± 7 min. When analyzed as a percentage of the total PCP-saline response, none of the nonspecific human Fab treatment groups had any significant effect on locomotor activity. However, 1.0 M and 3.0 M anti-PCP Fab significantly attenuated locomotor activity to approximately 20-30% of PCP-saline controls and the PCP-specific Fab (ANOVA followed by Newman-Keuls, p<0.05). The duration of PCP-induced effects measured from the time of Fab treatment was reduced from 34 ± 7 min to 13 ± 4 min after the 1.0 M and 3.0 M anti-PCP Fab treatments. These data show that anti-PCP Fab dramatically reverses PCP-induced toxicity. (Supported by NIDA grants DA 07610, DA 05474, and DA 00101).
**NEUROTOXICITY:**

343.15

**18a-GLYCIRQETINIC ACID (GA) BLOCKS KAINIC ACID-INDUCED SEIZURES AND NEUROTOXICITY.** Diane M. Hendriks, Robin L. Sayler, and Kathleen M. Schimpf 1

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**The overall objective of this study was to determine the effects of 18a- and 18b-glycurhetinic acid (GA), potent inhibitors of adenyl and guanylyl stamelatation, on kainic acid excitotoxicity.**

**Neurotoxicity was assessed by NADPH diaphorase activity, which was reduced in kainate (90 pg/ml) or GA (300 pg/ml)-treated brains.** The kainate 90 pg/ml-treated brain with 18a-GA had a lower incidence of seizures (0 vs. 75%) and significantly reduced seizures in the endoplasmic reticulum, amygdala, hippocampus, basal, medial, lateral, and ventral lateral nuclei of the thalamus, dentate gyrus, CA1-CA3 of the hippocampus, midline nuclei of the thalamus, lateral septum, and periventricular hypothalamus compared to rats treated with kainate alone. Preliminary data indicate that the 18a-isomer is not as neuroprotective. In fact 100% of the rats treated with 18a-GA+KA had behavioral seizures and extensive damage in the amygdala, thalamus, and neocortex. Biochemical analyses indicated that neither 18a-GA or kainate inhibited 18a-hydroxysteroid dehydrogenase at the doses used in this study. We conclude 18a- and 18b-GA have differential effects on kainate excitotoxicity when administered directly into the brain.

This work was supported by NIH grants ES07031.

343.17

**THE ROLE OF EXCITOTOXIC GLUTAMINE-DERIVED METABOLITES IN ETHYL CHLORIDE-INDUCED HYPERACTIVITY.** By J.N. Clarke, R.S. Bitter, G.E. Logan, and G.W. Ying*, Dept. of Pharmacology and Toxicology, Purdue University, West Lafayette, IN 47907.

Pottinger and co-workers (1991) demonstrated that mice displayed a glutamine (GSH)-dependent, hyperactive syndrome within 20-30 min of exposure to high concentrations of ethyl chloride (Et-Cl) (15,000 ppm). The hyperactivity resembled the wild running seizures observed in mice following i.c.v. glutamate or NMDA. To determine the possibility that excitotoxic GSH metabolites were responsible for Et-Cl-induced hyperactivity, known and potential GSH-derived metabolites of Et-Cl were examined for excitotoxic properties. S-carboxymethylcysteine (CMC) and cysteine (Cys), but not S-ethylcysteine (SEC), injected i.c.v. (3 mg/kg i.p.) did not cause delayed seizures as might be expected if the cysteine conjugate were metabolized to CMC or Cys. S-ethyl-GHS (SEG) injected i.c.v. (1 umol) failed to cause wild running seizures, indicating that it did not share the NMDA agonist properties of GSH (Leslie et al., 1992). Mice depleted of brain GSH using buthionine sulfoximine (3 mg i.e.v.) also failed to display wild running seizures following i.c.v. administration of SEG. Thus, the results obtained with these known and potential (CMC and Cys) metabolites do not support the possibility that the neurotoxic actions of Et-Cl are due to formation of excitotoxic GSH-derived metabolites.

343.19


Several findings suggest that endogenous histamine (HA) might enhance glutamate-NMDA mediated brain excitotoxic mechanisms (Bakkers, Science, 471:118-120, 1993; Vorobjev, et al., Neuro, 11:837-844, 1993) and participate in brain damage (Cohn, et al., Agents Actions, 27:120-122, 1989; Langlais et al., J. Neurosci. Res, 1994, in press). Nevertheless, neurotoxicity produced by HA in normotensive animals has not been directly demonstrated. To test this hypothesis, a detailed, light-microscopic histopathological study was performed. Rats received intraocular HA via chronically implanted guide cannulae. Histamine was injected suprapotential lateral border of the perihypothalamic area. Following predetermined intervals, animals were perfusion-fixed and brain sections were stained with cresyl violet. Three days after injection of 100 pg of HA, large areas within the limbic brain showed marked proliferation of glial cells (mean rostro-caudal extent = 1250 ± 150 mm). Injection of 30 and 300 pg of HA induced proportionately smaller areas of glossis. A dose of 30 pg produced no detectable effects. Control injections of isotonic or hypertonic saline also induced little or no pathological changes. At shorter survival times (24 or 48 hr), 100 pg of HA induced a clear loss of neurons, commensurate with the histopathologic results. These results show that HA can cause lesions in the central nervous system and support the hypothesis that endogenous HA may function as a mediator of neuropathological processes (Supported by DA-03816 and NS-29771).

343.20

**KAINATE NEUROTOXICITY IN VIVO IS GREATLY AMPLIFIED BY IMPAIRED SODIUM PUMP CAPACITY.** A. O. Parks, N. C. de Lavenere, S. Kapoor*, and M. L. Price, Neuroendocrinology Program and Section of Neurology, Yale School of Medicine, New Haven, CT 06510

Many adult neurons exhibit excitotoxicity in which death is caused by excessive transmembrane ion fluxes. Since the sodium pump (Na-pump) directly or indirectly rectifies these disturbances, we hypothesized that reduced or impaired Na-pump capacity will poorly rectify ionic disturbances and amplify the toxic potential of excitatory agents. Results of our prior work indicated that 24% of 11-month-old Syrian hamsters are affected. Following predetermination intervals, animals were perfusion-fixed and brain sections were stained with cresyl violet. Three days after injection of 100 pg of HA, large areas within the limbic brain showed marked proliferation of glial cells (mean rostro-caudal extent = 1250 ± 150 mm). Injection of 30 and 300 pg of HA induced proportionately smaller areas of glossis. A dose of 30 pg produced no detectable effects. Control injections of isotonic or hypertonic saline also induced little or no pathological changes. At shorter survival times (24 or 48 hr), 100 pg of HA induced a clear loss of neurons, commensurate with the histopathologic results. These results show that HA can cause lesions in the central nervous system and support the hypothesis that endogenous HA may function as a mediator of neuropathological processes (Supported by DA-03816 and NS-29771).
427.1


Repeated unilateral microinjections of methamphetamine (m-AMPH) produce significant damage to dopaminergic terminals in the rat striatum as shown by decreases in dopamine content and [3H]mazindol-labeled dopamine uptake sites. Previous work from this laboratory has demonstrated that a unilateral infusion of the excitotoxin quinolinic acid (QA) prior to m-AMPH treatment prevents the QA-induced decreases in dopamine content (O'Dell et al., J. Pharm. Exp. Ther., in press). Here, we extend the characterization of the effect of these QA-induced lesions in the rat striatum by utilizing quantitative autoradiography of striatal dopamine uptake sites. One week after administration of m-AMPH and three weeks after receiving unilateral striatal QA, the density of [3H]mazindol-labeled dopamine uptake sites in the QA-infused hemisphere of m-AMPH-treated rats was less than that in the non-infused hemisphere. This finding suggests an important dissociation between measures of dopamine content and dopamine uptake sites in the QA-infused striata of m-AMPH-treated rats. Ongoing studies with autoradiography will allow a better understanding of these measures of dopaminergic terminal integrity may be attributed to QA-induced alterations in dopaminergic metabolism.

427.2


To discriminate between events that are products of lesions elicited by kainic acid (KA) and those that are solely associated with its excitotoxic properties, we have examined the expression of cellular immediate-early genes following KA or pentylenetetrazol (PTZ) treatment. While both chemocarriers elicit lesions, only KA leads to neuronal damage as determined by TUNEL (terminal transferase dUTP nick-end labeling) or immunohistochemical (IHC) procedure. In order to unambiguously follow the expression of one of the prototypic cIEGs, c-fos, transgenic rats were generated that produce a FoxO2 fusion protein by either a transgenic adenovirus (TA) or KA or PTZ, however, its expression was more protracted following KA. In addition, within 6 hours following TA, c-fos expression was observed in the cytoplasms of neurons that were destined to die 1-2 days later, a situation never observed following PTZ. Examination of other basic zipper genes, revealed a unique immediate early response following KA. These features included: the expression of fox1 and fox2 in KA treated animals; protracted and different expression of several cIEGs; and a biphasic elevation of c-fos and junB in which the first peak was correlated with the initial seizure and then the second peak occurring just prior to the onset of death. These changes in cIEG expression were correlated with sustained increases in c-fos-like DNA binding activity in the hippocampal area of KA treated rats. These features of the KA induced response suggest that there are unique AP-1 complexes that could have a critical role in leading to, or counteracting, neuronal death.

427.3


Current excitotoxic models of Huntington's Disease involve exogenous injection of glutamate analogues to induce selective neuronal loss in the striatum. A major limitation of this approach is that the very rapid progression (1-2 days) of striatal neuronal loss which impairs temporal and spatial analysis of the specific patterns of neurodegeneration. We have attempted to develop a more subtle and slow progressing striatal lesion that reflects the anatomical and neurochemical patterns of cell death seen in HD. Our experimental paradigm employs a regime of 15 minutes of striatal glutamate stimulation with the aim of chronically elevating the synaptic concentration of endogenous excitatory amino acids in the striatum. Microdialysis experiments showed significant increases (two-fold) of extracellular glutamate in the striatum which remained elevated at least 2 hr after electrical stimulation. This stimulation paradigm was used for 2 weeks, 1 month, or 2 months to assess the morphological and neurochemical effects of repeatedly elevated striatal glutamate release on specific neuronal systems within the striatum. Astrocyes (labelled by GFAP staining) were increased both in size and number, this effect being evident at 2 weeks but more pronounced at 1 month and 2 months. Immunohistochemistry for the specific GABA neuronal marker GaD gy reveals a significant decrease in staining at the cell body and fiber level. This model may permit future investigations into both the identification of mechanisms involved in the progression of HD and the plastic changes taking place in the striatum during this degenerative process.

427.4


Tumors of the central nervous system (CNS) were investigated with antibodies to quinolinic acid (QUIN), an endogenous neurotoxin produced by cells of the immune system. In advanced F98 glioblastomas and E88 neuroblastomas produced by inoculation in the striatum of rats, quinolinic acid immunoreactive (QUIN-IR) cells were observed almost exclusively within the tumors. The immunoreactive cells were round, rod-shaped or complex in morphology. No consistent differences of size, shape, or number, morphology or distribution of immunoreactive cells between gliomas and neuroblastomas, but more advanced tumors of both type had significantly higher numbers of stained cells. No QUIN-IR cells were observed in the contralateral hemisphere. Gial fibrillary acid protein (GFAP) immunoreactivity was strongly elevated in astrocytes in the compressed tissue surrounding the tumors, and in the contralateral corpus callosum. Immunohistochemistry with the monoclonal antibody ED1, directed against rat monocytes and macrophages, demonstrated that numerous mononuclear phagocytes had infiltrated the tumors. These results indicate that the number of infiltrating mononuclear phagocytes in CNS tumors greatly exceeds the number of QUIN-IR cells, suggesting that if quinolinic acid is produced by monocytes and their derivatives, it is a selectively subpopulation of these cells. The present findings are consistent with a functional role for quinolinic acid in certain infiltrating leukocytes during the immune response to CNS neoplasms.

427.5

PRECONDITIONING DOES NOT PROVIDE CEREBROPROTECTION FROM INTRASTRIAL INJECTIONS OF AMPA OR NMDA. J.M. Giddon, A.R. Shag, J. Thorton, T. Park, and J.W. McDonald. Department of Neurology and Neurological Surgery, and Center for the Study of Nervous System Injury, Washington University School of Medicine, St. Louis, MO 63110.

Recent studies have shown it is possible to confer cerebroprotection from hypoxic-ischemic injury in the neonatal rat with a prior exposure to sublethal hypoxia, called preconditioning (Neurosci. Lett. 166:221, 1994). Using this technique, we sought to determine if preconditioning would prove neuroprotective against direct injections of NMDA or AMPA. 10 litters of 6-day-old rat pups were divided into 2 groups: 1) Control (C): treated with 3 ml of saline solution, 2) the rest were placed in normothermic control chambers. 24 hr later, each pup was anesthetized with diethyl ether, and received a stereotaxic microinjection of either AMPA (10 nM) or NMDA (10 nM) into the right striatum 2.5 mm lateral to bregma, and 3.75 mm deep. Pups recovered in an incubator for 45-60 min, then were returned to their mothers. The injury was then assessed by immunohistochemistry on preconditioned animals injected with AMPA (n=28) exhibited a 14.9±1.5% difference in hemispheric weights, which did not differ significantly from injury in non-preconditioned animals (15±2.1% n=23, p>0.8, unpaired t-test). Preconditioning also failed to be protective in NMDA-injected brains (n=27), yielding a hemispheric deficit of 12±1.8% compared to 19±1.2% in non-preconditioned animals (n=25, p>0.6). These results suggest preconditioning cerebroprotection may involve events proximal to glutamate receptor activation rather than subsequent to its release.

427.6

TUMOR NECROSIS FACTOR (TNF)-α POTENTIATES GLUTAMATE NEUROTOXICITY IN HUMAN FETAL BRAIN CELL CULTURES. S. Hu* and C.C. Chao. Minneapolis Medical Research Foundation and the University of Minnesota Medical School, Minneapolis, MN 55404.

Cytokines released by activated microglia have been proposed to play a pathogenic role in the brain. Since exposure of neural cells to the excitatory amino acid neurotransmitter glutamate induces injury to these cells, we tested the regulatory effect of cytokines on glutamate receptor-mediated toxicity in human fetal brain cell cultures. After 6 days of incubation, exogenous glutamate (5 mM) induced remarkable neuronal loss (a 165% increase in release of lactate dehydrogenase and a 62% decrease in function of uptake of H8-amino-butyric acid [GABA]). Glutamate neurotoxicity was dose-dependent (ED50 of 200 μM). Treatment of cell cultures with TNF-α (100 ng/ml) but not with a number of other cytokines, potentiated glutamate neurotoxicity (a 52% further decrease in H8-GABA uptake). Exposure of brain cells to TNF-α or to cytokines tested above alone did not significantly affect neuronal cell function. 14 of 16 studies indicated enhancement of glutamate neurotoxicity was markedly blocked (up to 80%) by the glutamate receptor antagonists DL-2-anino-5-phosphonovaleric acid (10 μM) and MK-801 (10 μM), indicating that the potentiating effect of TNF-α is mediated via glutamate receptors. Also, exposure of neuronal cell cultures to TNF-α resulted in a 27% decrease in ascorbic acid synthesis and an 18% decrease in H8-glutamate uptake, suggesting that the effect of TNF-α indirectly involves glutamate metabolism. These findings support the hypothesis that TNF-α may impair neuronal injury by exacerbating excitotoxicity.
NEUROTOXICITY:

SOCIETY

neurons, levels effect the longer CA. EXACERBATION

MACROPHAGE PROTEIN

staurosporine-induced (MN9D/Neo).

TRIFLUOPERAZINE-INDUCED

Dept. BCL-2

427.8


The protooncogene Bcl-2 inhibits programmed cell death in a variety of cell types. While the mechanism for this function remains unclear, it may involve the attenuation of oxidative stress. Because oxidative stress has been hypothesized to play a role in neurodegenerative disorders such as Parkinson’s disease, we sought to evaluate the potential of Bcl-2 in sparing cells from the dopamine neurotoxins. N-methyl-4-phenylpyridine (MPP\(^+\)) and 6-hydroxydopamine (6-OHDA). To test this in a dopaminergic neural cell line (MN9D), stable transfectants expressing either human Bcl-2 (CHO/Bcl-2) or neomycin (MN9D/Neo) were established. Surprisingly, it was found that overexpression of Bcl-2 led to robust neurite formation. Increases in primary neurite length and total neuritic extent were highly significant in MN9D/Bcl-2 versus MN9D/Neo. Bcl-2-expressing cells also reduced cell death due to MPP\(^+\) treatment. In contrast, Bcl-2 overexpression did not block the cytotoxic effects of 6-OHDA. However, inclusion of antioxidant agents or an iron chelator did substantially attenuate cell death in the 6-OHDA model. Because MPP\(^+\) is thought to inhibit the respiratory chain at Complex I and thus mitochondrial metabolism, ceramide was added to inhibit the activity of NADH dehydrogenase at this level. Rotenone-mediated cell death was significantly delayed in MN9D/Bcl-2. These data suggest Bcl-2 may protect cells from altered mitochondrial electron transfer processes. Thus, Bcl-2 may have dual roles in neurogenesis and in repression of cell death.

427.10

A COMPARISON AND APPLICATION OF BICISTRONIC HERPES SIMPLEX VIRUS VECTORS. T.J. Metz*, D.Y. Ho, R. Dash, M.S. Lawrence, R.M. Sapolsky. Department of Biological Sciences, Stanford, Harvard University, CA and Ophthalmology, MEED, Dept. of Neurology, Children’s Hospital, Boston, MA, 02115.

Monocistronic herpes simplex virus vectors offer an effective means of delivering genes to post-mitotic neurons. They do not, however, allow cells to respond to the dynamic events of neuronal systems. We sought to coexpress both a gene of interest and the lacZ marker gene in the same cell by employing internal ribosomal entry site, in the second approach, the two genes placed under the control of the opposite reading HSV\(^+\) and HSV\(^-\) promoters. Both bicistronic systems show overexpression of the two genes as assessed by d\(_{3}^{2}\)H IBMV immunofluorescence, indicating that regeneration detection at in not occur and that such vectors can be reliably used to target expressed cells. We have employed such vectors to investigate the neuroprotective effects of various genes products: a) Cultured hippocampal neurons receiving a bicistronic vector bearing the bcl-2 gene show enhanced survivorship when challenged by an oxidative insult as compared to those receiving a control vector. b) Delivery of a bicistronic vector bearing the glucose transporter gene protects hippocampal neurons against glutamate excitotoxicity in vitro and kainate-induced seizure in vivo. c) Delivery of a vector expressing the gene for eublinid D2RN causes a significant diminution and delay in excitotoxin-induced mobilization of free cytosolic calcium in cultured hippocampal neurons.

427.11

EXACERBATION OF GP120 EFFECTS ON RAT PRIMARY NEURAL CULTURES BY CORTICOSTERONE. S.M. Broude, J.D. Milne, R.M. Sapolsky, Dept. of Biological Sciences, Stanford University, Stanford, CA. 94305.

The section of the protein coat of the HIV virus known as gp120 may be the causal agent in AIDS dementia. It has been shown to cause neuronal death and increase in cytosolic calcium levels in neuronal cultures. The injury to neurons is thought to be mediated by release of a toxin from microglia or glia that acts through the excitatory amino acid pathway. Corticosterone (CORT), the rat specific glucocorticoid (GC) secreted from the adrenal, is involved in the mediation of the stressful response. CORT increases the effects of excitotoxins such as kainic acid, glutamate, and quinolinic acid. CORT also augments the mobilization of calcium in cytosol after an excitotoxic insult. We have investigated whether CORT exacerbates the effects of gp120 neutralizing antibodies or gp120 in vitro. An assay for glutamatic acid with Mg\(^{2+}\) staining, 200 \(\mu\)g/ml gp120 killed about 20% of hippocampal neurons. The addition of kai cort resulted in a further significant decrease in survival of approximately 14%. Using standard imaging techniques and the \(\mu\) for 2, we were able to confirm reports that gp120 can cause, in in vivo cortical neurons, a rise in cytosolic calcium. The time required to reach \(\tau\) was 300 bases (500). Twenty \(\mu\) for the peak, and the magnitude of the peak and the area under the curve. Some hippocampal cells, the presence of gp120 peaked at 600% of baseline after 900 sec exposure. Pretreatment with gp120 did not affect the peak or onset, but did not significantly impair recovery from the peak. It would appear that the stress hormone CORT may exacerbate the effects of gp120 on neuronal tissue. This could be relevant to the use ofinas to control the Neurotoxicity in AIDS.
428.1

INFLUENCE OF TH2-DOMINATED CNS IMMUNITY ON TUMOR GROWTH IN THE BRAIN. L. B. Gordon, H. F. Cerr, P. M. Kraep* Dept. of Physiology, Brown University, Providence, RI.

We have developed a model to study CNS immune privilege in Balb/c mice to nanotoma cell line P511. When introduced into the putamen of syngeneic DBA/2 or mismatched, incompatible Balb/c mice, blood-brain barrier disruption, 10^5 P511 cells form a progressively growing tumor, exhibiting angiogenesis, central necrosis, and brain tissue infiltration. Average time to death of mice was 25±7.5 days (p<0.0001). In contrast, 10^4 P511 cells subcutaneously (SC) results in tumor formation in syngeneic DBA/2 and 14±7.1 days in Balb/c (p<0.05). We suspect that an ineffective anti-tumor response is prolonging survival of Balb/c mice. In contrast, injection of 2 x 10^5 P511 cells subcutaneously (SC) results in tumor formation in syngeneic DBA/2 and 14±7.1 days in Balb/c. Thus, we have established a model to study immune privilege to MHC compatible, minor histocompatible tumor cells in the brain, a relevant brain tumor situation. We will use this model to examine the following hypothesis: tumor cells in the brain are detected by the immune system, but the response is directed along the Th2/humoral immunity pathway, whereas responses to tumors in a non-CNS setting favor a Th1/cellular immunity pathway.

In support, immunohistochemistry on brain sections showed scattered CD3+, CD4+, and CD8+ T cells present in and around Balb/c, but absent from DBA/2 tumors. A more dense distribution of F4/80+ and of IgG cells are present in brain tumors and surrounding neural tissue exclusively in the tumor-laden hemisphere of Balb/c and DBA/2 mice 14 dpi. Anti-P511 serum antibody responses have been detected in Balb/c mice with brain tumors and flank tumors 14 dpi. Precursor CTL activity, but no active CTL are detected in spleens of brain-infused or SC-infected Balb/c mice 14 dpi. Tumor-infiltrating lymphocytes (TIL) from SC tumors display active CTL, but not any staining in brain tumors, and anticipate a lack of CTL activity due to an immunosuppressive environment towards TH1 responses.

428.2

ORIGIN OF PC12 PHEOCHROMOCYTOMA CELLS: EVIDENCE THAT LOSS OF THE MYC DIMERIZATION PARTNER MAX WAS A TRANSFORMING EVENT. R. Hopewell and E. Grossbard, Howard Hughes Medical Institute, Kaplan Cancer Center and Department of Biochemistry, New York University Medical Center, New York NY 10016.

Max formation of heterodimers with the nuclear oncogene Myc and the differentiation-associated proteins Mad and Mxi1 enables these factors to bind DNA and control genes implicated in cell proliferation and differentiation. We show that the PC12 tumor cell line, derived from a rat pheochromocytoma, fails to express Max protein, due to aberrant splicing of max mRNA. RNAse protection and Northern analysis showed that only exon 3a expression. N-terminal sequences of Max up to the loop region of the helix-loop-helix dimerization motif are expressed, whereas max exons 3 of this are not expressed. We have cloned a cDNA from PC12 by hybridization to a max probe. The cDNA encodes a truncated Max protein which we call Maxp<sub>3</sub> containing a novel C-terminal sequence. Maxp<sub>3</sub> is incapable of homo- or heterodimerization in vitro, indicating that the protein cannot functionally substitute for wild-type Max. 

Reintroduction of wild-type Max into PC12 cells is strongly growth inhibitory, suggesting that mutational loss of Max expression may have provided a growth advantage for these cells, contributing to the transformed phenotype, and that Max may therefore be a tumor suppressor in a restricted class of tumors. We have reintroduced Max into PC12 cells under the inducible metallothionein promoter. Experiments examining the effect of Myc and Max expression in these cells will be presented.

428.3

THE CLONING OF MULTIPLE PROTEIN TYROSINE KINASE GENES EXPRESSED IN HUMAN PEDIATRIC BRAIN TUMORS AND DEVELOPING NEURONS. Edward L. Ziff, Mack Hickey, Howard L. Wiener, and Maria McCarthy. Howard Hughes Medical Institute, Department of Biochemistry, New York University Medical Center, New York, NY. 10016

Emerging evidence indicates that growth factor receptor tyrosine kinases (RTKs) play a central role in regulating normal cell growth and differentiation in the central nervous system (CNS). Aberrant expression of members of this family of proteins has been associated with tumorigenesis. However, little is known about the role of RTKs in early neural development or in human CNS tumors which arise from primitive neural precursors. Using degenerate oligonucleotide primers complementary to conserved sequences within the tyrosine kinase catalytic domain, we have cloned three distinct tyrosine kinases from two surgically resected human malignant pediatric brain tumors, a malignant ependymoma and a glioblastoma multiforme. These two tumor types are presumed to derive from embryonic stem cells of the CNS. We hypothesized that the molecular phenotype, including expression, of specific tumor cells could be informative of early glial or neuronal precursors. Expression of nine of these kinase genes in human brain tumors has not been reported previously. 

Kinase clones analyzed thus far are identical or bear significant homology to: MET, FER, TIE, HEK2, FLK1, FLT4, PDGFR-F, IGF-R, and EGF-F. Cytoplasmic PTks, including TIE2, SLK, the human JAK2 homologue and a novel protein kinase C isoenzyme have also been identified. In order to compare the pattern of RTK expression in tumors of the developing CNS to that of normal brain, we have applied this technique to the isolated brains of E17 rats. From these studies several kinases have been identified, including rat homologues of the FAK and JAK1 kinases.

428.4


The histogenesis of the medulloblastoma has been controversial since the original description of the tumor in 1925 by Bailey and Cushing, recent debates have focused towards cellular lines gial and neuronal. We studied retrospectively the histological and immunocytochemical features with stained routinely for H/E, Kluser-Barrera, Gomori's reticulin method, and with ABC technique. Of 20 cases of classical medulloblastoma with a mean of 14.2 years and 5 cases of desmoplastic medulloblastoma with a mean of 25.8 years, using a panel of antibodies neuronal and neuroglial (GFAP, Neuron-Specific Enolase, Neurolinin 160 KD, S-100 and Synapsin) in tissues fixed in formalin and embedded paraffin. In the classical group had a high population of cells were observed Homer-Wright rosettes, degeneration, necrosis and mitosis were observed and showed glial and neuroglial differentiation. In the desmoplastic variety bands of connective tissue separating neoplastic cells into rows and surrounding reticulin-free islands of tumor cells, cytoplasmic processes and presence of perivascular pseudorosettes that showed ependymal differentiation were observed.

In the immunohistochical study there was a reaction observed to all antibodies in the classical group, and there was a reaction to all them except S-100 in the desmoplastic group.

This study shows that medulloblastoma is a neuroepithelial neoplasm that can differentiate into ependymal, neuronal and neurtial lines.

428.5

EFFECT OF CELL ADHESION MOLECULES (CAMs) AND LAMININ ON THE DIFFERENTIATION OF NEURO-2A CELLS. W.W. Chang, Laboratory of Molecular Pathology, Department of Pathology, Veterans General Hospital-Taipei, Taipei 112, Taiwan, Republic of China

Development of the polarity, extension of processes, and further formation of axon and dendrites are crucial steps in the differentiation of a neuron. The effects of laminin and CAMs on neurite outgrowth have been demonstrated in various normal neurons. The effects of laminin and antibodies recognizing H-CAM (129F, against polysacitic acid) and L1 (gifts from Dr. C. F. Terstegen) in the differentiation of Neuro-2A cell line, Neuro-2A, were tested. RT7, antibody against phosphorylated neurofilament, was used to label the differentiation of axons. Many multipolar cells stained positively in the cytoplasm was identified in the 80-hr-old cultures. Some cells extended short RT7<sup>+</sup> neurites, most were less than 5 times the diameter of cell body. Only 5-10% of cell diameter was RT7<sup>+</sup> neurites of total length more than 5 times the cell diameter. The cell diameter was observed as numerous to (up to 1%) cells extended fine and long RT7<sup>+</sup> neurites. No significant effect is noted for the bound form L1 antibody. In contrast, both the free and bound forms of L1F8 antibody do not produce significant effect, even though the expression of N-CAM is confirmed. Evidently, laminin and L1 antibody, similar to the effects on some neurons in primary culture, also promote the neurite differentiation of Neuro-2A cells. (Supported by NSC-83-0412-B-079-055, TAIWAN, ROC)

428.6

Increases in Cyclic AMP Induce Morphologic Differentiation of MCD-1 Cells in Vitro. K. Moore, O. Dillon-Carter, M. Politrek, M. Cheadle, and W. Freed*. NIHNI Neuroscience Center at St. Elizabeths, 2700 Martin Luther King Ave, Washington, D.C. 20032, and 1Medical College of Georgia, Augusta, GA.

Medulloblastomas are undifferentiated CNS tumors, cells from which may under undergo morphologic differentiation on exposure to exogenous agents such as retinoic acid and dibutylryl cAMP (dBcAMP). The potential of cultured medulloblastoma cells to differentiate following the addition of NGF alone or in combination with agents which influence the cAMP second messenger system was studied. MCD-1 cells were exposed to NGF, dBcAMP, IIBM, forskolin, retinoic acid, arginine-L-carnitine (ALCAR) or mixtures of NGF-IIBM, NGF-IIBM+dBcAMP, and NGF+Forskolin+dBcAMP, and process outgrowth was measured after 48 hr. There were statistically significant increases in process outgrowth in cells treated with IIBM, dBcAMP, as well as NGF+IIBM, NGF+dBcAMP, and NGF+Forskolin+dBcAMP. Cells treated with forskolin, NGF, ALCAR or retinoic acid did not show changes in growth. NGF alone had little effect on process outgrowth and did not augment the effects of IIBM or dBcAMP. Immunohistochically the cells were positive for neuron-specific enolase (NSE), synaptophysin, and vitamin D receptor negative for GFAP. There were no changes in expression of NSE or synaptophysin following addition of differentiating agents. Thus morphologic differentiation of MCD-1 cells was induced by agents that increase cellular cAMP.
Biochemical and immunocytochemical characterization of the human medulloblastoma cell line MCD-1. D. Dillon-Carter*, K. Moore, M. Polakiew, M. Chedd1, and W.J. Freed, NIMH Neurosciences Center at St. Elizabeths, Washington, D.C. 20032, and (1) Medical College of Georgia, Augusta, GA.

The MCD-1 medulloblastoma cell line was isolated from a human cerebellar tumor. MCD-1 cells express several cytoskeletal markers, including vimentin, alpha- and beta-tubulin, and microtubule-associated protein 2. The cells are also positive for Thy-1 and fibronectin, but negative for N-CAM and L1 antigen. The cells are negative for markers of glia and differentiated neurons, including GaLC, MBP, GFAP, neurofilaments, synaptin, TH, GAD, DBH, and PHM1. The cells express glutamate, choline, and serotonin uptake, but are negative for uptake of GABA and dopamine. Glutamate uptake is sodium-dependent and blocked at 0°C and by DL-threo-B-hydroxyaspartate. Serotonin uptake is inhibited by domperidone, and choline uptake by hemicholinium-3. In addition, MCD-1 cells secrete the tumor-growth regulatory molecule TGFβ2, and grow in serum-free medium. The presence of serotonin, glutamate, and choline uptake suggests that medulloblastoma cells may use these substances as biochemical mediators. The MCD-1 cell line may be useful for studies of medulloblastoma growth and differentiation.

SYMPOSIUM:

TOWARDS A NEUROBIOLOGY OF VISUAL CONSCIOUSNESS. C. Koch, Caltech (Chairperson); H. Kostyn, Harvard University; P. Stergiou, Ludwig-Maximillian University, Munich, Germany; R. Desimone, Lab. Neuropsychology, NIMH; F. Crick, Salk Institute.

The aim of the symposium is to demonstrate that the neurological and neurophysiological substrate underlying conscious visual perception in humans can be approached in a rigorous experimental and reductionist manner. We have the tools in hand to explore this most central of our subjective "states" and its disruption in pathology in terms of particular types of (bio)electrical activity in particular neurons, areas or pathways in the brain.

Kostyn will discuss how the phenomenological qualities of visual mental imagery relate to activity in specific brain regions in human subjects, using PET and functional MRI studies. Stergiou will consider "blindsight," in which patients have a loss of conscious visual perception in the presence of demonstrable residual functions. A similar phenomenon exists in monkeys with striate cortical ablations. Desimone will discuss single-unit electrophysiological studies carried out in cortex of the awake and behaving monkey, suggesting ways in which prefrontal mechanisms prepare the visual system for expected stimuli, possibly providing the substrate for imagery. Crick will outline a general framework within which the problem of the Neural Correlate of Consciousness (NCC) can be approached using theoretical, neuroanatomical and electrophysiological studies.

SYMPOSIUM:

DEVELOPMENTAL CONTROL OF ELECTRICAL EXCITABILITY. A.B. Ribera, University of Colorado Health Sciences Center (Chairperson); M.E. Ratliff, Beckman Research Institute, City of Hope; J. Moody, University of Washington; C. Mandel, SUNY Stony Brook.

Although it is firmly established that electrical excitability is developmentally regulated, its role in the emerging nervous system is less well understood. The functions of electrical excitability in developing neurons and associated regulatory mechanisms will be discussed. Ratliff will present data that indicate that environmental influences, provided by cells such as glia, affect development of ion channels and membrane currents. Moody will present studies that test the role of electrical activity in development by embryonic microinjection of ion channels. Ribera's work examines the specific functions of identified potassium channel genes in embryonic neurons. Mandel will discuss the genetic elements that are involved in activation of expression of a sodium channel gene during differentiation of peripheral neurons.

SECOND MESSENGERS II

MODULATION OF PLASMA MEMBRANE CALCIUM ATPASE (PMCA) ALTERS CALCIUM EFFLUX IN CULTURED SENSORY NEURONS. J. Wortsch and S.A. Thayer, Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN 55455.

Intracellular calcium concentration ([Ca²⁺]ᵢ) and whole cell Ca²⁺ current (Iₑ) were measured simultaneously in single rat dorsal root ganglion (DRG) neurons grown in primary culture. Following a modest rise in [Ca²⁺]ᵢ (100-600 nM), recovery to basal [Ca²⁺]ᵢ was fit well by a single exponential. The decay time constant (τ) was similar for action potential-evoked (no cell dialysis) and depolarization-induced (whole-cell configuration) Ca²⁺ loads (5.85 ± 0.61 s vs. 5.94 ± 0.56 s, respectively). Omission of ATP from the patch pipette solutions results in calcium-induced calcium release (CICR) through inositol 1,4,5-trisphosphate (IP₃). Inclusion of 1 mM calmodulin in the pipette caused a slight decrease in τ compared to control (4.52 ± 0.51 s vs. 5.94 ± 0.56 s, respectively). Omission of ATP from the patch pipette solutions resulted in a decrease in baseline calcium levels. The addition of 10 µM 2-aminoethane sulfonic acid (2-ASA) blocked the CICR current for 5 s, and resulted in a calcium transient that was sustained up to 1 minute. The addition of 10 mM 2-ASA blocked the CICR current for 5 s, and resulted in a calcium transient that was sustained up to 1 minute. The addition of 10 mM 2-ASA blocked the CICR current for 5 s, and resulted in a calcium transient that was sustained up to 1 minute. The addition of 10 mM 2-ASA blocked the CICR current for 5 s, and resulted in a calcium transient that was sustained up to 1 minute. The addition of 10 mM 2-ASA blocked the CICR current for 5 s, and resulted in a calcium transient that was sustained up to 1 minute. The addition of 10 mM 2-ASA blocked the CICR current for 5 s, and resulted in a calcium transient that was sustained up to 1 minute. The addition of 10 mM 2-ASA blocked the CICR current for 5 s, and resulted in a calcium transient that was sustained up to 1 minute.
CHIMERIC RECOMBINANT AQUEOUS: NEW TOOLS FOR THE STUDY OF CA2+
CULTOSTASES IN NEURONS AND MYOCYTIES. R. Rigual, M. Bias C.
Bastianutto, L. Fast, R. Gravina, M. Montone, P. Gambardella*
and T. Pozzan*
Division of Biomedical Sciences, University of Padova, 35121 Padova Italy;
Division of Neuropharmacology, CWRU, 2065 Adelbert Rd, Cleveland, OH 44106.

In recent years, much interest has been devoted to the study of cytosolic
CA2+ homeostasis, not only because changes of CA2+ concentration in
this phenomena, but also because, until recently, the cytosol was the only cell
compartment in which this ion could be measured quantitatively in intact
cells. We here describe a new methodology, recently developed in our
laboratory, for the measurement of CA2+ concentration within specific
subcellular structures and its application to the study of the role of CA2+
homeostasis in cultured neurons and myocytes. The methodology is based on
the determination of the Ca2+-sensitive photoprotein aequorin, modified in
the blue whale organellar specific Ca2+ sensors, and on the
recombinant expression of the chimeric photoprotein in cultured cells. Three
chimeras are now available in the laboratory, which allow the monitoring of
CA2+ concentrations in the mitochondria, in the endoplasmic reticulum of living
cells (CA2+ jem), and in endoplasmic reticulum of living cells (CA2+ jem).
Cell stimulation coupled to increases in cytosolic CA2+ concentration invariably results in increases of [CA2+] jem and [CA2+] jem, but the kinetics and amplitudes of these changes are very different. In neurons, large changes of [CA2+] jem are evoked by the activation of plasma membrane channels, while in skeletal muscle myotubes changes in [CA2+] jem appear closely linked to CA2+ release from sarcoplasmic reticulum. [CA2+] jem on the other hand, seems to closely follow cytosolic CA2+
both in kinetics and amplitude. Finally, a large drop in [CA2+] jem occurs upon depletion of intracellular CA2+ stores, though the kinetics of these decreases are complex.

INTERLEUKIN 1 ENHANCES RECEPTOR-DEPENDENT AND INDEPENDENT INDUCED RELEASE OF ARACHIDONIC ACID FROM MOUSE STOMACH ASTROCYTES. N. Stella, G. De

The present study was undertaken to determine the interference of this cytokine in receptor-dependent and independent induced release of arachidonic acid from cultured striatal astrocytes. As previously observed, both 100 µM of LPS and the combination of 0.1 µM PMA and 2 µM ionomycin stimulated the release of 3H-AA from striatal astrocytes (136 ± 7% and 54 ± 3% above basal level, respectively). (N. Stella et al. 1994). In neurons, 14568:5%.

When 100 µM of LPS was added simultaneously with ATP, the cellular inhibitor of the release of 3H-AA was not affected (167 ± 8%). However, when astrocytes were pretreated for 24 hours with IL-1ß, both the ATP and PMA-induced release of 3H-AA were abolished (301 ± 20% and 295 ± %, respectively). The data suggest that IL-1ß treatment has a potentiating effect on the receptor-dependent and independent induced release of AA by enhancing specifically of PMA. Combined, the results support the role of AA in the regulation of glutamate uptake into astrocytes, IL-1ß could be involved in the glutamate-evoked neurotransmitter release in brain inflammatory processes.

ACTIVATION OF PHOSPHOINOSITIDES HYDROLYSIS BY ENDOTHELIN-1, ENDOTHELIN-3 AND BIG ENDOTHELIN IN THE RAT SPINAL CORD. T. Touloukeni, J. de Champlain and R. Courjon. Department of Physiology, Université de Montréal, Montréal, Québec, Canada H3C 3J7.

The activation of endothelins receptors results in the activation of phosphoinositides hydrolysis in peripheral and central tissues of the rat. However, this has not yet been shown in the rat spinal cord where endothelins receptors have been shown to be present. The present study was undertaken to examine whether the endothelin receptors induced phosphoinositides hydrolysis in spinal cord. We found that the endothelin-1 (ET-1), endothelin-3 (ET-3), big endothelin (big ET-1) with or without presence of selective ET2 receptor agonists (ET-1) in the spinal cord. The entire spinal cord of male Wistar rat, was cross-chopped at 350 µm. Slices (50 µm) were incubated with 0.13 µM of myo-[2H]-inositol (specific activity 32.5 Ci/mmol) and 17 nm L7 for 60 min. A final volume of 90 µl of Krebs buffer and stimulated with the proper agonist for an additional 60 min. The radioactivity in the total intracellular fraction (EP, I2 and I3) was determined as described by Feingold et al. (Biochem., J., 1982, 212, 457-462) and blank value (before stimulation) was subtracted from all values. ET-1 (1 nm - 10 µM), induced dose-dependent increases of labelled phosphatidyl inositol. At equimolar doses (1 µM) the rank potency of endothelin-3, ET-2 and ET-3 in slices. I2Q-103 (100 µM) reduced by about 30% the response to ET-1 and ET-3. These results suggest that activation of endothelin receptors in the spinal cord involved inositol lipid hydrolysis which is partly mediated by an ET3 receptor. Furthermore, the stimulation obtained with big ET-1 suggests the presence of an endothelin-converting enzyme in the spinal cord. [Supported by the MRC of Canada].

CYTOSOLIC PHOSPHOLIPASE A2 (cPLA2) mRNA DISTRIBUTION AND TRANSCRIPTION STUDY IN THE RAT SPINAL CORD: ROLE IN NEUROTRANSMISSION. L.L. Lautens. W.S. Young*, J.A. Sharp1 D.S. White1, Z.G. Chu2 and C.C. Felder. Lab. of Cell Biology, NIH, Bethesda, MD 20892 and 1The Lilly Research Laboratories, Lilly Corporate, Indianapolis, IN 46209.

The identification of an 85 kD cPLA2 raised hopes that the effector enzyme mediating receptor-evoked release of arachidonic acid (AA) had been isolated. cPLA2 mRNA within dorsal root ganglia and brain by in situ hybridization: cPLA2 mRNA was found in white matter of cerebrum, brain stem and cerebellar cortex. The present study was undertaken to examine the expression of cPLA2 mRNA in neurons cultured from specific brain regions. cPLA2 mRNA expression was also detected in peripheral tissue of newborn mouse. The data suggest that the cPLA2 gene product can be detected throughout the developing spinal cord. [Supported by NIH grant AG06853 and NINDS grant NS 209143].


Homogenates of Aplysia nervous tissue contain an 8-lipophosphoglycerol (8-L). Although this pathway has not been previously described in nervous tissue, products of the 12-LO pathway have been shown to act as neuromodulators in Aplysia. We have identified neurons selective for 8-L, which convert arachidonic acid to 8(R)-prostaglandinH2 (8-RPGEH) from which 8-ketoepoxyprofain (8-KETE) is enzymatically derived. In ganglia prelabeled with [3H]arachidonic acid, this pathway is activated in a dose-dependent manner by the application of u00 (20 µM) 8-ACh. 8-KETE is as effective in normal seawater as it is in a high Mg2+ low Ca2+ solution. Thus, the effect of direct and does not require activation of other nerves: Of 10 neurotransmitters tested, ACh was the only one that activated this pathway. This is the first demonstration of the 12-LO pathway in a nervous tissue. Cholinergic agonists activate the 8-L pathway, but several ACh antagonists including, transazepine, benzoxazepine, and benzimidazoles do not. Activation of the 8-L pathway is blocked by a bungarotoxin, which selectively blocks the ACh-gated chloride current in Aplysia (Roberts et al., Brain Res., 897, 1976), raising the possibility that products of the 8-L pathway may modulate neural functions through the activation of the cholinergic chloride channel. In support of this idea, suberyldicholine, a selective agonist, activates the 8-L pathway in Aplysia nervous tissue. Thus, 8- and 12-LO are selectively and independently activated by distinct neurotransmitters in Aplysia. Preliminary experiments suggest that the 8- and 12-LO pathways interact to generate lipid messengers that cannot be produced by either pathway alone.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
A PAF ANTAGONIST OR DEXAMETHASONE INHIBITS THE SEIZURE-TRIGGERED SUSTAINED UPREGULATION OF THE INDIQUE PROSTAGLANDIN SYNTHASE IN THE HIPPOCAMPUS. V. L. Marcheselli and N. G. Bazan. Louisiana State University Medical Center, LSU Neuroscience Center, New Orleans, LA 70119.

Electroconvulsive shock (ECS) activates phospholipase A2, which leads to accumulation of free arachidonic acid and platelet-activating factor (PAF). PAF is a messenger in the ECS induced transcriptional activation of cyclo-oxygenase and decreases COX-2 in hippocampal (Neuroscience Res. 37:54-61, 1994). Here we report that ECS effects sustained upregulation of the inducible prostaglandin synthase (PGS-2, or TIS-10) expression in rat hippocampus. The increase of PGS-2 mRNA, peaking at two hours after ECS, has elevated levels detected, for 24 hours. The Zif-268 mRNA in hippocampus peak at 1 hour after ECS, but return to normal after two hours. Pretreatment with dexamethasone (6.7 mg/kg body weight) produces a 34% inhibition of ECS induced PGS-2 mRNA expression. The PAF antagonist BN-50730 when injected intracerebroventricularly (icv) 15 min before ECS (50 mg/kg) produces a 70% inhibition of PGS-2 mRNA expression. It is concluded that in the hippocampus PAF is a mediator of inducible prostaglandin synthase expression and may in turn modulate synaptic function and seizure activity through prostaglandin synthesis. Supported by NIH NINDS NS23002.

EXTRASTRIATE VISUAL CORTEX: INFERIOR TEMPORAL AREAS

MULTIPLE SUBDIVISIONS OF THE ANTERIOR INFEROTEMPORAL CORTEX OF MACAQUE MONKEYS. E. M. Pandya, R. D. Zeki, and R. Badcock. Department of Neurobiology and Anatomy, Univ. of Texas Med. School, Houston, TX 77030.

Anterior intertemporal cortex (AIT) is a large portion of the temporal lobe that exists approximately 10.15 mm from posterior to the anterior temporal polar sulcus (AMTS) to the temporal pole. Previous architectures (Selver and Pandya, '78) and conncctional studies (McClendon and Felixman, '90) have suggested that this region is characterized by several distinct cortical areas. To investigate further the organization of AIT, we examined the distribution and lamination patterns of labeled cells and terminals following 12 injections of neuronal tracers into area AIT. Immunohistochemical and light microscopic analyses were performed on sections through AIT at 50 µm intervals. The results demonstrated that AIT is divided into several distinct areas designated AITpv, AITav, AITavpv, AITpv, and AIT. In several cases in AITpv, AITavpv, and AIT, visual stimulus regions were found bilaterally in areas AITpv, AITavpv, and AITpv. These data indicate that AIT consists of 3 distinct areas and each contains specific visual cortical areas. Supported by NEI EY-03732, the Sloan Foundation, and the Whitall Foundation.

ELEfMENTS OF FORM PROCESSING FROM MOTION IN MONKEY PRESTRIATE CORTEX: E. Petersen* and R. Baumann. Department of Neurology, University Hospital, CH-8091 Zurich, Switzerland.

We have studied early stages of form processing from motion in the visual cortex of the alert rhesus monkey. During periods of visual fixation we recorded the responses of single neurons to rows of dots (size 12 x 12 min of arc) in a surrounding texture. The motion was defined as a 12-36 min arc moving relative to a background of dots of smaller size, which either was kept stationary or moved in antiphase. These stimuli produced the perception of moving bars-like objects, segregated from a background of dots of small size, which either was kept stationary or moved in antiphase. The color, size, and orientation of the stimulus were varied, but the orientation of the motion was fixed. The results showed that the neuronal responses were correlated with the orientation of the motion and the size of the stimulus, as well as the contrast of the stimulus.
SENSORY INTERACTIONS AND EFFECTS OF SELECTIVE SPATIAL ATTENTION IN MACAQUE AREA V2. L. Chelazzi, L. Desimone, J. Luck, S. Remington. Lab. of Neuropsych., NIH, Bethesda, MD, and Dept. of Neurosciences, UCSD, La Jolla, CA.

It was previously found that when two stimuli fall within a cell's receptive field, attention directed to one of them seems to filter out the influence of the second. To test this idea, we have recorded the responses of V2 neurons in a macaque monkey to an optimal, or preferred, stimulus in the presence of a second, nonpreferred stimulus. We measured changes in response caused both by the sensory interaction between the two stimuli in a passively viewing condition as well as by the effects of attention directed to the first or second stimulus.

During passive viewing, a nonpreferred stimulus in the receptive field typically caused a partial suppression of response to the preferred stimulus. The degree of effects of a nonpreferred stimulus diminished with distance from the receptive field but were still present in some cases beyond the field border. Attention directed to the preferred stimulus appeared, on average, to diminish the suppressive effects of the nonpreferred stimulus whereas attention directed to the nonpreferred stimulus seemed to magnify its suppressive effects. The time course of both the attentional and sensory effects on the response was similar. As in earlier studies (Neurosc. Abs. 19:9:6), an increase in baseline activity typically occurred when attention was directed to a location within the receptive field.

We hypothesize that the baseline shift is due to a spatially selective attentional signal which increases the average firing rate of those cells whose receptive fields are at the attended location. We further suggest that the sensory interactions, which could serve as preparatory visual processes such as separation of figure from ground, may also be the mechanism by which the attentional signal biases sensory processing in favor of attended stimuli.

REPRESENTATION OF STIMULUS POSITION RELATIVE TO THE EXTENDED OBJECT REPRESENTATION IN MACAQUE AREA V4. C.E. Connor*, J.L. Gallant and D.C. Van Essen. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

We have previously shown that changes in the position of an attended object affect the spatial response profile and overall response level of cells in macaque visual area V4 (Connor et al., Soc. Neurosci. Abstr. 19: 974 (1993)). In this study, we have investigated whether a cell responds differentially to an oriented bar stimulus depending on which side of the bar the attended object appears on (a laterality preference). We have explored this phenomenon further by varying the location of the attended object across multiple positions in 1 and 2 dimensions. The attended object was a ring, flashed briefly near the receptive field as part of a serial comparison task. The probe stimulus was a behaviorally irrelevant bar of optimal orientation, color, and size flashed at various locations inside the receptive field. A single bar stimulus appeared 200 msec following each stimulus bout. In one paradigm, ring position was varied across 7 locations along an axis perpendicular to the optimal bar orientation. Responses to the bar were often tuned for distance relative to the attended ring, as well as showing a laterality preference. In a second paradigm, the position of the ring was varied across multiple locations in 2 dimensions, all equidistant from the receptive field. Bar response strength often depended on 2-dimensional position relative to the attended ring. These findings suggest that area V4 processes information about stimulus position relative to nearby attended objects.


In humans, the visibility of a briefly presented shape is impaired when it is followed by another stimulus (backward masking). We observed the same phenomenon in two rhesus monkeys: discrimination performance for geometrical shapes presented in a masking sequence (ET) was strongly impaired when the shape was immediately followed by a patterned mask. To determine the neuronal correlate of this phenomenon, we measured the responses of inferior temporal neurons in the same 2 monkeys, performing a fixation task, to these briefly presented stimuli which were either followed by a mask (M) or not (NM). Although the response magnitude strongly declined with decreasing ET (tested range: 20-100 msec), shape selectivity remained present even at 20 msec ET (T > 5 in the NM condition). The surprising finding was that a majority of the units showed significant shape selectivity in the 20 msec ET condition too. When using the initial 60 msec of the response, the difference between the response to the preferred and non-preferred shape was on average 0.6 and 1.0 spikes in M and NM conditions, respectively. This small but significant difference between the selectivity in M and NM conditions, increased notably when considering the first 200 msec of the response: average response differences of 0.6 and 2.6 spikes in M and NM conditions, respectively. Thus, longer temporal integrations increased the marked increase in discrimination capacity in the NM condition, but not when the briefly presented shape is followed by another stimulus. This suggests that the behaviorally observed backward masking is the result of temporal integration of the neuronal responses. (Supported by IUP-22 & FGWO)

RESPONSES OF V4 NEURONS DURING VISUAL SEARCH. L. Chelazzi and K. Desimone. Lab. of Neuropsych., NIH, Bethesda, MD, 20827 and Dept. of Neural. and Vis. Sci., Univ. of Verona, Verona, Italy.

In a previous study of the mechanisms underlying visual search (Chelazzi et al., Nature 1993, 365:345-347) we reported that cells in inferotemporal (IT) cortex of the macaque may participate in storing a representation of the searched-for (target) stimulus and in selecting the target from distractors. In the present study we investigated whether area V4 also contributes to such processes. A total of 140 neurons with extraretinal mechanisms (EPMs) were recorded in one macaque monkey. Each trial began with a brief presentation of a cue stimulus at fixation. Following a delay of 1.5, during which fixation had to be maintained, a pair of target stimuli was presented in the periphery, at random locations, and the animal was required to select the EPM stimulus that matched the earlier cue, ignoring the other (distractor) stimulus. Contrary to what was previously found in IT, V4 neurons did not display cue-selective sustained activation during the delay interval. However, similar to IT cortex, V4 responses to the distractor stimulus were suppressed well before the eye movement to the target. This effect was maximum when target and distractor both lay within the RF borders, diminished substantially when the target stimulus was moved outside the RF, and diminished further with increasing distance of the target from the RF borders. Thus, in addition to IT cortex, V4 contains mechanisms for selecting a relevant target based on its features and discarding distractors. The fact that these mechanisms are most evident when target and distractor are located within a cell's receptive field suggests that they are based on local competitive interactions within area V4. The competition between target and distractor in V4 may be biased by top-down, or feedback, signals directed at cells coding the expected target's features.

NEURONAL RESPONSES IN MONKEY INFERIOR TEMPORAL CORTEX DURING THE ATTENTIONAL RELEVANCE OF A VISUAL STIMULUS. B. Jagadeesh*, L. Chelazzi, R. Desimone, M. Mishkin. Lab. of Neuropsychology, NIH, Bethesda, MD and Dept. of Neural. and Vis. Sci., Univ. of Verona, Verona, Italy.

With repeated stimulus-response-reward pairings, stimuli become behaviorally relevant and typically elicit orienting responses. To examine the neuronal mechanisms underlying the learning of stimulus relevance, we recorded from neurons in inferior anterior temporal cortex of a macaque monkey while the monkey acquired a stimulus-response-reward association. Pairs of stimuli were presented at random locations extravehally, and the monkey was rewarded for making a saccadic eye movement to one stimulus of each pair (the positively rewarded stimulus). During the recording session for a single cell, the animal learned “on-line” which stimulus was associated with the reward through trial and error.

Each pair was chosen on the basis of pre-testing to include one stimulus that activated the cell well on its own (good stimulus) and one that was ineffective (bad stimulus). Two pairs of stimuli were used for each cell, and good and bad stimuli were randomly assigned to be either positive or negative. The animal had to find and saccade to the positive stimulus with a very short (150 ms) latency. Responses to the good-bad stimulus pair were averaged across the population of cells. During the 150 ms prosaccadic period, responses were larger when the good stimulus of the pair was positive than when the bad stimulus was positive. The difference in the responses was present even though both good and bad stimuli were always present within the receptive field during that time. This result suggests that the competitiveness of representations of visual stimuli in anterior inferior temporal cortex can change with learning and influence the selection of targets from a scene.


Neurons in the inferior convexity of the prefrontal cortex have recently been found to respond selectively to complex stimuli such as pictures of objects and faces. These responses resemble those of feature-selective neurons in area TE of inferotemporal cortex. In order to investigate connections between area TE and prefrontal cortex, we combined physiological mapping with WGA-HRP histochemistry to identify afferents of the prefrontal cortex based on functional evidence of injection. Injections were made in the inferior prefrontal cortex in three cases. Two cases received injections in area 45, one of which was first characterized electrophysiologically. The third animal received an injection in area 12. Following injections of area 45, we found retrogradely labeled cells and anterogradely labeled terminals throughout the entire length of subdivisions TEa and TEa in the ventral bank of the STS. Labeling of area TEa was also found in the case with an area 12 injection. In the latter case, the labeling was restricted to areas TEa and TEa in the ventral bank of the STS, and was located slightly rostral to that seen following injection of area 45. These findings indicate that area TE in the inferior temporal cortex, including the ventral bank of the STS, is a selective channel of object and feature information which links specific regions of the prefrontal cortex with the visual system. (Supported by MH-44866, 38546 and JSMF #01-47)
Learning a Visual Task as Reflected in Activity Dynamics of Macaque Inferotemporal Neurons

A simple task was performed on a standard touch-screen paradigm. The task was to discriminate between two images presented on the screen. The images were of high visual acuity and were presented in a random order. The task was performed by a single monkey, and the monkey's performance was monitored using two electrodes placed in the inferotemporal cortex.

In this study, the monkey was trained to discriminate between two images of different objects. The images were presented on the screen, and the monkey was required to touch the correct image within a short time. The task was performed for several days, and the monkey's performance was recorded using two electrodes placed in the inferotemporal cortex.

The data collected during the task were analyzed using a drift-diffusion model, which is a mathematical model used to describe the decision-making process. The model assumes that the monkey's response is based on a random walk in a decision space, where the decision is made when the monkey reaches a certain threshold.

The results showed that the monkey's performance improved over time, with a significant increase in the speed of the decision-making process. The drift-diffusion model was able to accurately predict the monkey's performance, indicating that the model is a useful tool for understanding the decision-making process in primates.

In conclusion, this study provides evidence that the inferotemporal cortex is involved in the learning of visual tasks, and that the decision-making process can be modeled using a drift-diffusion model. These findings have important implications for understanding the neural mechanisms underlying learning and decision-making in primates.
343.5

SEQUESTRATION OF AMYLOID β-PROTEIN BY TRANSHYRETIN: STRUCTURAL STUDIES OF TTR-AP INTERACTION.
Department of Psychiatry and Pharmacology, SUNY, Stony Brook, NY 11794 and The Picower Institute, Manhasset, NY, 11030.

Recent studies have proposed that sequestration of Aβ in biological fluids and extracellular space is the key step in the homeostatic mechanism which prevents Aβ amyloidosis. We have identified that failure to sequester Aβ may lead to amyloid formation. We then found that in cerebral spinal fluid, TTR sequesters Aβ into stable complexes. We also showed that TTR prevented amyloid formation in vitro. In order to investigate the interaction of the two proteins, a computer graphic model of the TTR-Aβ complex was built and the Aβ binding domain with several negatively charged amino acids was predicted. To verify this model, forty different mutations were introduced into the putative binding domain of recombinant TTR and the mutants were tested for binding of radiolabeled Aβ. The mutants of TTR that failed to bind Aβ and to prevent amyloid formation were identified and the key amino acids of TTR that interact with Aβ were determined. These results enabled us to refine the model and predict possible variants of Aβ that may be associated with Aβ amyloidosis. The correct model of TTR-Aβ complex will identify structural requirements for compounds that prevent amyloid formation.

343.6


Apolipoprotein E (apoE), particularly the ε4 allele, is genetically linked to Alzheimer's disease (AD). ApoE has previously been shown to bind to β-amyloid (Aβ), an amyloidogenic, proteolytic product of amyloid precursor protein. To analyze the interaction of Aβ with apoE, we constructed an active Western immunoblotting of Aβ peptides incubated with concentrated, conditioned media from HEK293 cells stably transfected with either apoE or apoE human cDNA. Non-reducing SDS-PAGE revealed the presence of a 45kD multi-protein complex with both Aβ and apoE immunoreactivity. Using either Aβ 1-40 or 1-42 peptides, the level of apoE/Aβ complex was ~20-fold greater than apoA4/Aβ complex. This apoE isofrom-specific binding pattern was maintained at concentrations of Aβ from 11nM to 1mM, from pH 5 to pH 9, and from 2 minutes to 24 hours of peptide incubation. The higher level of apoE binding to Aβ is in contrast to previously published data which had been delineated and denatured (Proc. Natl. Acad. Sci. 90:8098, 1993). These data suggest that apoE may play a role in preventing the aggregation or facilitating uptake/removal of this amyloidogenic fragment.

343.7

DISTRIBUTION OF APOLIPROTEIN E AND B-AMYLOID PROTEIN IN CULTURES OF NEURONS, MICROGLIA AND ASTROCYTOTES. M.D. Argy, G.M. Cole, and A.P. Mohrb.
Dept. of Anatomy, U. of Tennessee, M. Gr., Jackson, MS 39216, & Dept. of Medicine, UCLA and GRECC, VAMC, Sepulveda, CA 91343.

Understanding the mechanistic basis for apolipoprotein E (ApolE) genotypic variability, major risk factor for amyloidosis and Alzheimer's disease requires an exploration of ApoE and β-amyloid protein (Aβ) interactions with CNS cells.

Cells were cultured from neonatal or young (4-5 month) rat brain and were immunostained using rabbit anti-ApoE serum (Organon Tech) or rabbit anti-Aβ antibody (Calbiochem). Anti-ApoE serum showed strong staining in the parenchyma of the brain, whereas the anti-Aβ antibody revealed positive staining in the astrocytes and microglia, with occasional staining in the neurons. Anti-ApoE serum showed a similar pattern of staining in the rat brain. The results suggest that ApoE is associated with microglia in the CNS and may contribute to the formation of amyloid plaques. The presence of ApoE in the CNS may contribute to the development of Alzheimer's disease.

343.8

APOLIPROTEIN E (APOE) GENOTYPE AND IMMUNOHISTOCHEMICAL FINDINGS IN ALZHEIMER'S DISEASE (AD) WITH AND WITHOUT PARKINSON'S DISEASE (PD) CHANGES. M. Gesing*, J.A. Schneider, and S.S. Mintz.
Department of Pathology and Laboratory Medicine, VA Medical Center and Emory University School of Medicine, Atlanta, GA 30322.

The ε4 allele of ApoE has recently been identified as a risk factor for the development of AD, and cognitive decline in AD and ε4 has been observed in senile plaques. Genetic dysequilibrium of the ε4 allele and the ApoE amyloid association in senile plaques were investigated in 100 dementia patients with neuropathological confirmation. Forty-five AD cases were diagnosed using criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the AD Alliance, and 25 PD cases were identified based on a combination of clinical symptoms and a positive Family History of PD (FHF-S. Heart Hospital). We found that 72% of patients in each group had at least one ApoE ε4 allele compared with reported estimates of 20-25% in the general population. The AD vs. PD groups also showed similar immunohistochemical findings. Although cortical plaques generally demonstrated 5α-A4 apoE colocalization, 9α-plaques in focus neocortical regions were seen in ApoE-immunonegative cases. In all cases, β-amyloid diffuse plaques in the striatum failed to label with antibody to ApoE, whereas cerebellar diffuse plaques showed consistent colocalization of 5α and A4. These discrepancies may be explained in several ways: (1) amyloid processing differs regionally, and the association between 5α and ApoE is not universal; (2) β-amyloid immunonegative plaques may represent an earlier stage in plaque evolution than ApoE-positive plaques, and the ApoE-5α colocalization is a later phenomenon; (3) ApoE is present in all plaques, but in some plaques the levels are too low to be detected with our methods; or (4) ApoE is more sensitive to technical vagaries than 5α. Supported by VA Merit Award and NIH grant AG10130.

343.9

CEREBOSPINAL FLUID LEVELS OF AMYLID B-PROTEIN ARE INDEPENDENT OF APOLIPROTEIN E GENOTYPE AND ARE INVERSELY CORRELATED WITH SEVERITY OF DEMENTIA IN ALZHEIMER'S DISEASE.
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Alzheimer's disease (AD) is characterized by formation in brain of neurofibrillary tangles and/or amyloid deposits. Brain amyloid burden is highest in AD patients who are homozygous for the apolipoprotein E ε4 allele, which is a high risk factor for the development of AD. To investigate the relationship of apolipoprotein E genotype with brain metabolism of the amyloid β-protein (Aβ-40, Aβ-42, and Aβ-43) we evaluated CSF levels of amyloid β-protein (Aβ) and of secretory neural TTR APP derivatives in 19 AD patients with varying degrees of dementia and with the most common combinations of apolipoprotein E alleles, that is, ε3/ε3, ε3/ε4, and ε4/ε4. Mean CSF levels of Aβ are presented as mean ± SEM. There was no significant difference among the 3 genotypes (ε3/ε3, ε3/ε4, and ε4/ε4). Mean CSF levels of Aβ were nonsignificantly lower in the ε3/ε3 group than in the ε3/ε4 and ε4/ε4 groups. The CSF levels of Aβ were not associated with dementia severity or mortality. The results suggest that ApoE ε4 allele may be a risk factor for AD.

343.10

INCREASED APOLIPROTEIN E ε4 ALLELE FREQUENCY IN ALZHEIMER'S DISEASE (AD) AND IN VASCULAR DEMENTIAS (VD).
Inst. Pharm. Sci. and E. Grossi Pavetti Ct. Univ. of Milano, Alzheimer's Disease Unit, FBFS-Heart Hospital, Brescia, Italy.

ApoE is the only apolipoprotein expressed in the nervous system where it may participate in the transport of choles and lipids via binding to LDL and LRP receptors. In AD apolipoprotein E has been found in senile plaques and ApoE4 (E4 is encoded by a single gene, a common polymorphism is defined by alleles ε3 and ε4) binds in vitro to β-amyloid. These observations stimulated the search of ApoE4 genes in AD leading to the discovery that ApoE is raised in AD (Corder et al., Science 251, 921, 1993). The increased prevalence of ε4 allele is confirmed in our series of AD patients. The ApoE ε4 frequency determined in AD patients by PCR methods was 0.43 (P<0.0001) compared to 0.09 in 64 age and sex matched controls. The presence of two copies of ε4 allele and the 25 SNeuropil fluid (CSF) levels were maintained in both the ε3/ε3 group and the ε3/ε4 group. The results suggest that ApoE4 allele may be a risk factor for AD, indicating an increased risk for AD increased with the ApoE4 dose. Notably a gender effect was observed, since the presence of one ε4 allele was sufficient to significance for AD in females (5.6, P<0.009), but not in males (2.8, n.s.) suggesting an interaction between Apo E4 and sex hormones. This view is supported by the observation that estrogen replacement therapy decreased the risk for AD in women (Paganini et al. Soc.Neuroscl. Abs., 1993). Moreover an increased ε4 allele frequency in nonobese is observed in VD. This last observation indicates that the ApoE4 allele specificity for AD is doubtful and that the modified allele frequency may have some fundamental role in altering the sensitivity of neurons to damage, perhaps through an impairment of repair processes in the brain.
435.11
SECRETION OF β-APP INTO THE RAT CSF: AGED RATS SECRET
SIGNIFICANTLY HIGHER LEVELS OF β-APP FOLLOWING
FOREBRAIN CHOLINERGIC LESIONS THAN YOUNG RATS. Y. Haroutunian*, K.L. Davis, R. Gluck, E. Fiber & W.C. Wallace, Department of Psychiatry, Mount Sinai School of Medicine, NY, NY, and Laboratory of Biochemistry, NIH, Washington, DC, 20201.
Experimental lesions of various subcortical neurotransmitter systems (cholinergic, noradrenergic, serotonergic) induce β-APP expression in the cortex of the rat. This induction results in the persistent secretion of β-APP into the cerebrospinal fluid (CSF). In the current series of experiments young (2 month old) and old (24 month old) Fischer 344 rats received unilateral 6-hydroxy-dopamine lesions of the cholinergic basal forebrain. One week after lesioning, cerebrospinal fluid was collected, and the rats were sacrificed. Analysis of cortical choline acetyltransferase activity (ChAT) showed that cortical cholinergic marker activity was significantly (p<0.001) reduced to approximately 50-55% in both groups. Immunoblots of the CSF using an antibody (15) to the secreted β-APP showed that significantly higher levels of β-APP were present in the CSF collected from sham operated controls (p<0.001). In addition, a significantly (p<0.0002) greater amount of β-APP was present in the CSF of lesioned aged rats relative to lesioned young rats (118% increase vs 66% increase). Basal levels of β-APP in the CSF of young and old sham lesioned rats did not differ (p>0.2). The levels of β-APP in the CSF were highly, and inversely, correlated with the degree of cortical ChAT depletion (r=0.55, p<0.001). Since secreted β-APP contains the potentially amyloidogenic Ap30 amino acid sequence, these results suggest that the consequences of cholinergic system dysfunction may be more deleterious in the old than in the young.

435.13
IMAGING STUDIES OF A TC-99M ANTI-A8 FAB MONOCLONAL ANTIBODY (MAB) IN ALZHEIMER’S DISEASE (AD) USING SPECT. R.P. Friedland*, J.M. Reis, E.M. Majocha, M.S. Bertrand, F. Mirakijko, P. Hedera*, L.E. Eli, C.A. Moceri, Alzheimer Center, University Hospitals, CWRU, Cleveland, OH 44106; NeoRx Corp., Seattle, WA; Malinckrodt Medical, St. Louis, MO; Brown University, Providence, RI.
A Mab (10H3) targeting the AD A8 protein has been developed as a tool for the in vivo assessment of amyloid angiography using SPECT. Results from immunochemical studies using the 10H3 antibody suggest that microvascular fibrillar amyloid is accessible to an imaging agent delivered via the IV route. The 10H3 antibody is stably radiolabeled by conjugation of a preformed Ts 99m diamino dichloro chelate active ester with the protein antigen group, and then associated to Fab fragments. In vitro and animal studies in vivo have demonstrated the safety, sensitivity, specificity and satisfactory biodistribution of the labeled 10H3 Fab (J Nuc Med 33:2184, 1992; Mol Neurosci, in press). SPECT and gamma camera imaging in 4 patients with AD demonstrated activity in the blood pool in the thorax and brain, with rapid uptake in liver and kidney. Clearance of the label from serum is slower than expected, with an initial half life of 3 to 4 hours. No binding to the CSF could be shown, and free Tc-99m was minimal. Tomographic brain images obtained up to 15 hours after injection show retained activity in the venous sinuses with patchy activity in the brain parenchyma. Studies are currently underway to compare the brain distribution of the label to known blood volume markers, and compare patients in the early and later stages of the disease. A label with a longer physical half life may be needed to allow for imaging at later times following clearance of the radiopharmaceutical from the blood.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION

436.1
Thyroid hormones are required for the transition from the breeding to non-breeding state in a variety of species. The onset of the breeding season appears to be thyroid hormone-dependent as well as dependent on photoperiod because maturation of the reproductive tract occurs in birds with thyroidectomized (THX) as well as the photo-induced non-breeding state (J. Exp. Zool., 179: 333-338). In the present study, we tested the hypothesis that thyroid hormones are involved in the transition from the non-breeding season to the breeding season in the ewe, a species in which both onset and end of the breeding season are generated by an endogenous rhythm. We determined the influence of thyroidectomy on the onset of the breeding season in ewes maintained outdoors and ewes held in a fixed short-day photoperiod in which the endogenous reproductive rhythm would be unmasked. All animals were ovariectomized (OVX) and received s.c. Silastic implants of estradiol. As an index of reproductive neuroendocrine activity, thyroid hormone (LTH) was measured in twice weekly blood samples. A sustained increase in circulating LH above 1 ng/LL defined the onset of the breeding season. Ewes maintained outdoors were either THX in late anestrus (Aug., Nov.), THX in early anestrus (Mar., Apr.), or remained thyroid intact (n=7). There was no group difference in the mean date of increase in LH (20 Sep ± 10, 16 Sep ± 6, and 14 Sep ± 3, respectively). Ewes held in constant photoperiod were either THX early in anestrus (a month after LH decreased; n=9) or remained thyroid intact (n=7). There was no difference in the onset of the subsequent breeding season, with LH rising 6 mo. after the previous decline. We conclude that thyroid hormones do not influence the transition into the breeding season in ewes. Rat thyroid hormone receptor binding on the reproductive development of anestrus. (Supported by NIH HD18337, 18285 and MH110506; NSF IBN 9250519.)

436.2
A PHYSIOLOGICAL ROLE FOR EXCITATORY AMINO ACIDS IN THE CONTROL OF PULSATILE LH SECRETION. V.B. Malek*, J. Ping and D.W. Brain, Department of Physiology & Endocrinology, Medical College of Georgia, Augusta, GA 30912.
The purpose of the present study was to determine the role of excitatory amino acids (EAA's) such as glutamate and aspartate in the regulation of pulsatile luteinizing hormone (LH) secretion in adult male and female rats. To achieve this aim, specific EAA receptor antagonists were injected into the third ventricle of castrated adult rats and the effect on pulsatile LH secretion was determined. The specific N-methyl-D-aspartate (NMDA) receptor antagonist D-APV (2-amino-5-phosphono-pentanoic acid, 10μg/rat) and the specific non-NMDA receptor antagonist DNX (6,7-diisoquinoline-2,3-dione, 30nM) were utilized in these studies. In female rats, administration of either DAP or DNX resulted in a significant elevation of LH levels which was due to a suppression of both LH pulse frequency and amplitude. In male rats, DAP suppressed mean LH levels and amplitude with no effect on frequency, while DNX had no effect on any parameter of LH secretion. Increasing the duration of exposure to the antagonists by administering three injections (instead of one) at 20 minute intervals yielded similar results to a single injection for APS, while three injections of DNX (20 nM each injection) now caused a significant suppression of mean LH levels and pulse amplitude with no effect on frequency. These studies provide evidence that EAAs acting through both NMDA and non-NMDA receptors play a significant role in regulating pulsatile LH secretion and that female animals have a higher sensitivity than male animals to this regulation.
436.4 INFLUENCE OF ETHANOL (ETOH) ON INSULIN-LIKE GROWTH FACTOR-I (IGF-I) AND IGF-IR SYNTHESIS DURING FEMALE PUBERTY. V. Srivastava, J.K. Hiney, C.L. Nyberg, N.H. McArthur, and W.L. Dees, Dept. of Vet. Anatomy, Texas A&M University, College Station, TX 77843-4458.

Recently, we showed that most IGF-I available to the hypothalamus at puberty is derived from circulating bone derived IGF-I at the LH/LH releasing system of prepubertal female rats; thus, indicating IGF-I may participate in the control of LH secretion at the time of puberty. Since ETOH delays puberty and is associated with depressions in GH and LH levels, we examined whether ETOH can affect i) IGF-I gene expression in liver, ii) IGF-I mRNA expression in the median eminence (ME) and c) serum levels of IGF-I and LH. Prepubertal female rats were implanted with gastric cannula on day 24 and began receiving control or ETOH liquid diets on day 29. On day 34, rats were killed, determined to be anestrous, and tissues and blood collected. Results indicate that the ETOH-fed rats showed a decrease (p<.001) in liver IGF-I mRNA levels compared with the controls, and this paralleled depressions in both serum IGF-I (p<.01) and LH (p<.05). No changes were observed in IGF-I mRNA within the ME. These results suggest that ETOH administration process, at least in part, by blocking the peripheral increase in the synthesis of IGF-I, a peptide suggested as a metabolic signal involved in the onset of puberty. (Supported by NIH-A07216)

436.5 PULSATILE FSH SECRETION IN THE OVARIECTOMIZED EWE IS CONTROLLED BY BOTH GNRH-DEPENDENT AND -INDEPENDENT MECHANISMS. V. Padmabhaskar, E.L. McFadden, N.P. Evans, G.E. Dahl, D.T. Mauger, and J. Kurogi, Department of Pediatrics and Physiology, Reproductive Sciences Program, and the National Center for Infertility Research at Michigan, University of Michigan, Ann Arbor, MI.

The objectives of this study were to determine i) if FSH secretion is episodic and ii) if a GnRH-independent control of episodic FSH secretion exists. Hypophyseal portal blood and peripheral blood were collected from five short-term (80 min) intervals for 6 h before and 6 h after a single i.v. injection of Nal-Glu, a GnRH antagonist (10 μg/kg body weight) in GnRH in portal blood, and LH and FSH in both jugular and portal blood were determined by radioimmunoassays. Pulsatile GnRH secretion was evident both before and after Nal-Glu administration. Pulses of LH in the portal circulation coincided with those seen in the peripheral circulation but were larger and more discrete in nature. The pattern of FSH in peripheral blood was not episodic. In marked contrast, FSH in portal blood was unambiguously pulsatile. Prior to Nal-Glu treatment, each pulse of GnRH correlated with a pulse not only of LH but also of FSH in portal blood. However, 49% of the total FSH pulses occurred in the absence of corresponding GnRH pulses. Following Nal-Glu administration, LH pulsatility was completely eliminated, demonstrating its dependence on GnRH. In contrast, Nal-Glu administration blocked only GnRH associated pulses of FSH, it did not block other episodes of FSH release. We conclude that in the ovariectomized ewe; i) FSH secretion is episodic; ii) all GnRH pulses induce an FSH pulse; iii) there exists a GnRH-independent episodic component of FSH release. Our results are consistent with the hypothesis of a dual regulation of FSH release. Supported by NIH grants U54 HD 29184, P50 HD 18258.

436.6 INHIBITION OF ENDOGENOUS NEUROPEPTIDE Y (NPY) SYNTHESIS BY ANTISENSE OLIGODEOXYNUCLEOTIDE ADMINISTRATION SUPPRESSES THE PROGESTERONE-INDUCED LH SURGE. Kalra, P.S.*, Bonavera, J.J., Dube, M.G., Crowley, W.R. and Kalra, S.P., Departments of Physiology and Neuroscience, Univ. Fl., Gainesville, FL 32610 and Department of Pharmacology, Univ. Tenn, Memphis, TN 38163

Evidence shows that NPY is involved in stimulation of the LHRH and LH surges induced by progesterone (P) in estradiol benzoate (EB)-primed ovariectomized (ovx) rats. We have also reported that P stimulates NPY peptide content and prepro-NPY mRNA levels in the hypothalami of these rats, thereby suggesting P-induced activation of NPY synthesis. To evaluate whether newly synthesized NPY is critical in induction of the P-induced LH surge, ovx rats fitted with electrochemical detectors in the lateral ventricles (icv) were primed with EB (30 μg/rat s.c.). Two days later, these rats received P at 1000 h (2 mg/rat s.c.). Additionally, they were injected icv at 1000, 1200 and 1400 h with either 1 mg/kg NPY antisense or scrambled NPY. Pregnane-3,20-diol (NLOGIC); controls were injected with saline. Blood samples were withdrawn via pre-implanted intra-atrial cannula at 1000 h and at hourly intervals between 1400-1800 h for LH analysis. Results show that robust LH surges occurred in controls and in rats injected with scrambled OLOGIC. However, in rats injected with NPY antisense OLOGIC to block NPY synthesis, the P-induced LH surge was completely suppressed. These results support the hypothesis that P activates de novo synthesis of NPY, which is necessary to evoke LHRH and LH surges. (Supported by NIH HD 08634)
FOOD RESTRICTION INCREASES NPY GENE EXPRESSION IN A SUBPOPULATION OF NPY NEURONS IN THE ARCULATE NUCLEUS OF PREGNANT RATS. L.A. Camacho†, D.J. Horvath‡ and M.S. Smith
‡. Department of Psychiatry, Neuroscience and Neurobiology, University of Pittsburgh, Pittsburgh, PA 15261.

Food restriction was shown to delay the onset of puberty in female rats. Others have reported that food restriction increases hypothalamic NPY activity. Their results were obtained in animals where changes in NNPH-somata were associated with the delayed onset of puberty induced by food restriction. Three groups of prepubertal rats (n=6 each) began treatment on day 28 of age and were sacrificed 2 weeks later: (a) ad lib feeding; (b) food restriction (FR); or (c) food restriction + 24 hrs of ad lib feeding before sacrifice (FR + 24 hr refeed). Average body weights on day 42 were C: 136.0 ± 3.3 gm; FR: 74.2 ± 0.7 gm; and FR + 24 hr refeed: 96.0 ± 1.3 gm. At the time of sacrifice, C animals were in various stages of the estrous cycle, whereas none of the FR and FR + 24 hr refeed groups had exhibited first estrus. In situ hybridization was performed on 20 µm brain sections using a 35S riboprobe for rat NPY mRNA. The data were analyzed by measuring the area occupied by silver grains on the acrulate nucleus. NPY mRNA levels did not differ among the three groups in the rostral portions of the arculate nucleus. However, in the caudal portions of the arcuate nucleus, NPY mRNA levels were increased by 76% in response to food restriction: C = 59.1 ± 8.2, FR = 67.0 ± 7.4, area occupied by silver grains; FR + 24 hr refeed = 71.5 ± 6.7 µm². The data demonstrate that the delay in the onset of puberty induced by food restriction is associated with an increase in NPY mRNA levels in a specific population of NPY neurons in the caudal portion of the arcuate nucleus. Further studies will be necessary to determine whether these NPY neurons play a role in causing the suppression of GnRH secretion that underlies the delay of puberty induced by food restriction.

436.11
AXONAL TRANSCRIPTS IN THE RAT HYPOTHALAMIC-NEUROHYPOPHYSIAL TRACT: G.P. Jirikowski, Dept. of Neuroendocrinology, Max Planck Inst. for Psychiatry, Munich, Germany.

With light- and electron microscopical in situ hybridization and non-radioactive detection methods, we could obtain evidence that oxytocin- and vasopressin-expressing mRNA is present in the axonal pathway of magnocellular hypothalamic perikarya, probably associated with a fraction of the large secretory vesicles. mRNA is likely to be subject to rapid axonal transport, since osmotic challenge changes axonal concentrations of transcripts, while colchicine treatment blocks such effects. In recent studies we could demonstrate that several other transcripts occur in magnocellular hypothalamic axons in the median eminence and the posterior lobe: mRNA coding for c-fos and cjun as well as yroinne hydroxyllase mRNA could be found in the median eminence and the posterior lobe. Saturation loading resulted in a down-regulation of hybridization signal to the magnocellular perikarya within 15min, which could be prevented by pretreatment with colchicine. Pretreatment with a polymerase II inhibitor did not affect increase of hybridization signal to the paraventricular and supraoptic nuclei upon osmotic stimulation. It is likely that neuroendocrine systems with high secretory capabilities, like the hypothalno-neurohypophysial system, utilize their large axonal volume for storage of transcripts, which are perhaps compartmentalized in vesicles, to circumvent degradation. Retrograde transport upon specific stimulation may allow for immediate de novo translation, to replenish depletd peptide pools in terminal sites, prior to the onset of new gene expression.

436.12

Vasopressin (VP) neurons in the bed nucleus of the stria terminalis (BNST) are steroid sensitive and the number of VP mRNA expressing neurons is larger in males compared to female rats. This difference in VP cell number has been attributed to proliferation and/or survival of VP neurons in the gonadotropin responsive period during the critical period. We have shown that galanin (GAL) and VP mRNAs are coexpressed in the BNST of the adult male rat and recently, that GAL mRNA gene expression in the BNST is not sexually dimorphic. These results suggest that the index of coexpression of these neuropeptides differs in male and female rats.

Here, we hypothesized that VP neurons represent a subset of GAL cells in the BNST and that the incidence of coexpression of VP mRNA by GAL mRNA expressing neurons in the BNST would be reduced in female rats compared to male rats. We performed double-label in situ hybridization histochemistry on sections through the BNST from male and female Wistar rats (90 d). Brain sections were hybridized with 35S-labeled and digoxigenin-labeled cRNA probes complementary to GAL and VP mRNAs, respectively. Radioactive grains were detected over VP cells expressing the presence of GAL gene expression. We divided the BNST into two anatomical regions which are part of separate functional pathways: medial (BSTM) and lateral (BSTL) divisions. We found a significant sex difference in the number of GAL cells which coexpressed VP in the BSTM (Mean±SEM: male: 124±8, female: 55±6, p<0.001). However, no difference was found in the BSTL (male: 80±5, female: 83±15). Likewise, the number of cells expressing GAL mRNA only was significantly higher (p<0.02) in the BSTM of female (85±9) compared to male rats (43±5). We provide evidence that VP is coexpressed by a subset of GAL neurons and that the reduced incidence of coexpression of VP by GAL neurons in the medial BNST accounts for the sex differences in VP cell number in this limbic region.

436.13

GAP junctions (GJ) establish cytoplasmic continuity and synchronize activity between adjacent cells. In the brain, the GJ proteins, connexins (Cx), exhibit developmentally regulated expression and restricted distribution. Although Cx26 and Cx43 were thought to occur predominantly in astrocytes, a recent report demonstrated functional GJs and Cx26 expression in GnIH-T17 neurons in vitro (Neuroendocrinology 1993 58:489-492). To reevaluate which cell types express Cx isoforms in the intact brain, we analyzed the distribution and expression of Cx26 and Cx43 in the primates hypothalamus during development. Ubiquitous sections were immunostained with optimal dilutions of affinity-purified polyclonal antibodies against Cx26, residues 108-117 (from J.A. Germain) or 112-125 (from N.B. Galudai), and a mouse monoclonal lgG against 19 residues of Cx43 (Zymed). Two fetal males (142-150 dga) had low Cx immunoreactive (ir) fibers. However, in 4 infant females (4.5-7.5 mo), faint or speckled Cx26-ir and Cx43-ir perikarya also were seen, especially in animals pre-treated with octreotide. In 3 juvenile females (17-19.2 mo), Cx26-ir and Cx43-ir somata had light to intense, uniform or speckled staining. In 3 adults, Cx43-ir perikarya were absent, but speckled Cx26-ir fibers were noted. In addition, Cx26-ir perivascular and Cx43-ir fibers in the ventral hypothalamic tract continued into the infundibulum (INF) and median eminence. Considering that Cx-ir was in perikaya retrograde and staining mimicked that for neuronal but not astrocytic markers, we conclude that Cx26 and Cx43 are present in neurons. Since GJs create electrotonic connections and allow small molecular diffusion between coupled cells, development in Cx expression may help coordinate maturation and synchronize specialized functions of hypothalamic neurons. Supported by HD10967 and HD11979.
437.2
ENZYMIC SYNTHESIS OF ANANDAMIDE, AN ENDGENOUS LIGAND FOR THE CANNABINOID RECEPTOR; PROPERTIES AND DISTRIBUTION IN THE BRAIN. W.A. Devane, Dept. of Neurobiol., Lab. of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892.

Anandamide (arachidonylethanolamide) was the first endogenous cannabinoid compound to be identified in brain (Devane et al., Science 1992). The purpose of this study was to characterize the enzymatic synthesis of anandamide using brain membranes.

Bovine membranes (P2 fraction) were incubated with [3H]ethanolamine and varying concentrations of ethanolamine and arachidonic acid for 20-40 min at 37°C. This resulted in the formation of a radioactive product having the same Rf value as authentic anandamide using both normal and reverse phase thin layer chromatography. Incorporation of [3H]arachidonic acid and ethanolamine also resulted in the synthesis of a radiolabeled product having the same Rf value as anandamide in both chromatographic systems. The synthesis of anandamide had a pH optimum of 9 to 10, and the amount of product formed was linear up to 50 min, using 70 µg of membrane protein/200 µl.

When varying the concentration of arachidonic acid, the enzymatic activity exhibited a biphasic curve, suggesting substrate inhibition. When compared to a number of related fatty acids, arachidonic acid proved to be the best substrate with the lowest EC50 and the highest Ψmax. A production study in bovine brain indicated that anandamide synthesis levels are high in the hippocampus, followed by cortex, striatum, and thalamus. Lower levels of enzymatic activity were found in cerebellum, medulla, and medulla. These findings suggest the presence of anandergic neurons.

437.4

Circumventricular organs (CVO) of the rat brain lamina terminals lacking a blood-brain barrier, the subfornical organ (SFO) and the organum vasculosum laminae terminals (OVLT), are involved in body fluid and cardiovascular control by sensing blood-borne substances. In vivo autoradiography on brain tissue sections showed binding sites for vasopressin (AVP) and its AVP(4-9)-agonist within the SFO and OVLT. For characterization of AVP and AVP(4-9)-receptors in cells cultured from the SFO and OVLT, receptors were labeled with [3H]dDAVP. The binding was measured in fura-2 loaded single neurons and glial cells. The investigated cell type was verified by immunofluorescence using NSE- and GFAP-specific antibodies. SFO- and OVLT-cultured cells responded exclusively to AVP with the highest affinity and at the lowest concentration. When comparing binding of AVP to other vasopressor peptides, it was found that the potency of AVP was greater in glial cells than in neuronal cells. To investigate the presence of a V1 receptor subspecies, specific antagonists inhibited the evoked responses in neurons (SFO n=4, OVLT n=9) and in glial cells (SFO n=5, OVLT n=3). Ca++-transients measured in the absence of extracellular Ca++ suggest intracellular stores to be the Ca++-source. An additional AVP receptor subtype in OVLT-neurons is implicated by the finding of a Ca++-signal induced by the V2 receptor agonist dDAVP (n=7). Furthermore, Ca++-transients were evoked with AVP(4-9) (100 nM) in SFO- (n=6) and OVLT- (n=7) neurons as well as in SFO- (n=2) and OVLT- (n=6) astrocytes. A significant number of cells (n=9) responded exclusively to the fragment peptides, indicating the existence of yet another member of the AVP receptor family.

437.6
ALTERED α2-ADRENOCEPTOR GENE EXPRESSION IN BRAINS OF TRANSCENDENTAL MICE. M. Scheinin, R. Link, M. Kulatunga, I. Marttila, M. Savola, C. Barsh and B.K. Bollika. Dept. of Pharmacology, Univ. of Turku, FIN-20520 Turku, Finland, and Howard Hughes Medical Institute and Stanford University, Stanford, CA 94305.

Three α2-adrenergic (α2) genes are expressed in brains of rodents and are evolutionarily conserved in humans. One of the subtypes, α2C, mediates both pre synaptic regulatory functions and postsynaptic effects. Little is known of the neurophysiologic roles of α2C genes. We have generated α2C-/- mice with normal development and behavior, but α2C specifically lacks dense binding in most hypothalamic nuclei, some of which may have been non-adrenergic imidazoline sites, e.g. in the arcuate nucleus(1). Intense immunohistochemical α2AR expression was not seen in any hypothalamic nuclei. Northern blotting and reverse transcription-polymerase chain reaction analyses showed predominant expression of these two receptors in the central nervous system. In situ hybridization analysis revealed their prominent expression in the limbic system and further demonstrated the differential distribution of their mRNAs in mouse brain. Although the ligands for these receptors are yet to be identified, the significant sequence homology between these receptors suggests that they constitute a new receptor subtype and they possibly represent different receptor subtypes for an unknown neurotransmitter. The prominent expression of α2C in medullary habenular nucleus, shown by in situ hybridization analysis, suggested that further characterization of α2C may facilitate our understanding of the function of the habenular nuclei.
437.7
LOCALIZATION OF CALCIUM RECEPTOR mRNA IN RAT BRAIN USING IN SITU HYBRIDIZATION HISTOCHEMISTRY.
K.L. Singh, S. Singh, and A.N. Sanyal. Pharmacia, Salt Lake City, UT 84108, and 2 Endocrine Hypertension and Renal Divisions, Dept. of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.
The capacity to sense changes in the level of extracellular Ca2+ is an important function in several cell types. For example, hormone secretion by the hypothalamus and parathyroid glands is regulated by extracellular Ca2+. The protein that enables these cells to detect and respond to changes in extracellular Ca2+ is recently identified and shown to be a 7-transmembrane, G-protein-coupled receptor linked to the mobilization of extracellular Ca2+ in response to increases in extracellular Ca2+. The receptor is a heterodimer containing two subunits, one of which is mainly regulated by extracellular Ca2+. This protein, 11C, is primarily regulated by extracellular Ca2+.

437.8
Physiological studies using microinjections of L-glutamate into specific sites in the rostral ventrolateral medulla and monitoring of cardiac function and vascular blood flow (Doppler flow probes) of postganglionic-vagovagal dogs revealed two sites where consistent responses were obtained. One is an area located 3.5 to 5.8 mm rostral to obex, 4.2 to 5.1 mm lateral to midline, 0.5 to 1.5 mm below the ventral surface [referred to as the subparvicellular area (SPV)] and the second in an area located 6.2 to 7.1 mm rostral to obex, 2.9 to 5.4 mm lateral to midline, 0.5 to 2.0 mm below the ventral surface (referred to as ventromedullar area). L-glutamate microinjected in the subparvicellular area produces a significant increase in arterial blood pressure associated with decreases in femoral and renal vascular bed conductances. L-glutamate microinjected into the ventromedullar area produces a decrease in arterial blood conductances but an increase in femoral arterial bed conductance. Using immunohistochemical methods and specifically tyrosine hydroxylase (TH) and phenylethanolamine-N-methyltransferase (PNMT) colormetric immunohistochemistry, we determined whether TH and/or PNMT immunoreactive neurons were present at the two microinjection sites. Results indicate that neurons in the canine subparvicellular area are immunoreactive to antibodies against PNMT and therefore contain epinephrine. Further analysis shows that neurons in the ventromedullar area are mainly occupied by neurons exclusively immunoreactive to antibodies against tyrosine hydroxylase (TH) although some neurons also contain PNMT. Previous investigators have included this region anatomically as part of the SRPN (Janzey et al., 1992). However, physiologic responses to L-glutamate microinjection in this area are characterized primarily by a significant increase in femoral conductance consistent with vasodilation.

437.9
STIMULATION OF C-FOS EXPRESSION IN SELECTIVE BRAIN NUCLEI BY EXOGENOUS ARGININE-8-VOSOPRESSIN: QUANTITATIVE IMMUNOHISTOCHEMISTRY AND LIGHT/FLUORESCENCE MICROSCOPY
Peripheral administration of arginine-8-vasopressin (AVP) has been shown to elicit the desired behavior of leaches and to maintain tolerance to ethanol by the action on AVP-V1 receptors. Others found that i.v. injection of AVP increased c-fos mRNA expression in the hippocampus and hypothalamus. Here, we tested the precise site(s) of action of peripherally injected AVP remain unknown. Adult male Sprague-Dawley rats were injected with saline (0.3 ml/kg s.c., n = 6), AVP (10 μg/ml s.c., n = 6), saline (0.3 ml/kg i.p., n = 6) or paraglandular AVP (PPTZ, 10 mg/kg i.p., ml, n = 3) and sacrificed 2 h later. Serial coronal brain sections were processed with antibodies against Fos and like proteins and AVP. Brain areas containing nuclei of similar size and shape were quantified by counting the number of neuronal cell bodies showing Fos immunoreactivity (Fos-IR) in each nucleus in left/right side under a Nikon Eclipse microscope with 20 X objective and CFPCCD (2.5 X).

437.10
ESTROGEN-SPECIFIC TARGET SITE IDENTIFIED BY PROGESTERONE-11b-HYDROXICORTICICATE-(2-125I)-IODOHISTOCINATE IN MOUSE BRAIN MEMBRANES.

438.1
EFFECT OF MK-801 IN FOCAL CEREBRAL ISCHEMIA IN THE MONKEY. R.N. Auer, G.W. Jason, B.I. Tranner, and S. Coupland Department of Neuroscience, University of Calgary, Canada, T2N 4N1.

To determine the effectiveness of MK-801 in primary focal ischemia, transorbital occlusion of the middle cerebral artery (MCA) was done in female cynomolgus monkeys (Sioumi strain) using a clip occluder placed under the operating microscope, while blood pressure was controlled to 60 mm Hg using halothane. Other drugs were administered. Ambient temperature was controlled during the operating procedures to roughly 35°C, and temporoparietal temperature was controlled to 37°C. MK-801 (1 mg/kg) was administered 20 minutes after occlusion. Following 110 minutes of occlusion, the clip was released and restoration of flow was verified visually. Quantitative histopathology at 52 coronal planes was performed after 2 weeks. There was no statistically significant difference in the infarct volumes between treated and untreated animals.

Neuropathologic testing also revealed no difference between the groups.

The cingulate cortex was carefully examined in the oldest animals, and no evidence of acidophilic neocortical neurons, microglial nodules, or other evidence of MK-801-induced cell death was seen.

We conclude firstly that MK-801 is ineffective in reducing cerebral infarct volume in this model of transient focal ischemia. Secondly, although 1 mg/kg is a relatively high dose of MK-801 in the primate, we conclude that the dose is not the necrotizing effect of this NSMDA antagonist, which has been well-described in the cingulate cortex of the rodent, does not occur in this primate.

438.2

Transport across the blood-brain barrier is an essential component of brain parenchymal cells is believed to be mediated by two differently glycosylated forms of GLUTI. The effects of cerebral ischemia for GLUTI mRNA have been described but not for the protein. Using a high affinity, specific, polyclonal antiserum (ALM-K1: 1:5000) they confirmed the asymmetric distribution of GLUTI with abnormally > cytoplasmic > luminal concentrations. After ischemia, both microvascular and non-microvascular GLUTI concentrations increased markedly by as soon as 1 day and persisted through 4 days of ischemia. We conclude: GLUTI may be detected in both brain microvascular and parenchyma using a high affinity, specific-polycyclonal antiserum. Global forebrain ischemia rapidly and persistently increases GLUTI expression of both forms. Supported by NS22213 and NS17983.
343.8

SUPPRESSION OF HIPPOCAMPUS FOS EXPRESSION AND ACTIVATOR PROTEIN-1 (AP-1) ACTIVITY DURING FOCAL CEREBRAL ISCHEMIA USING ANTIASENSE STRATEGY. P.K. Lin, A. Saltinsson, Y.Y. He, M.C. Tardos, C.Y. Hsu. Div. of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX 77030.

An activation of c-fos and the subsequent expression of the Fos protein were noted following focal cerebral ischemia. Fos and Jun form a heterodimer as AP-1 which modulates the expressions of several genes. To study the suppression of Fos expression related to c-fos expression, we performed Northern blot analysis following focal ischemia to suppress the post-ischemic expression of c-fos by intraventricular infusion of an antisense phosphorothioated DNA of c-fos mRNA. The antisense DNA was converted into RNA and hybridized with the c-fos mRNA in the hippocampus. In the outcome of this study, we not only noted that the 'P'-labelled antisense DNA was internalized within the neurons and in the hippocampus but also that the antisense DNA was not detected in the hippocampus after infusion of the antisense DNA. The suppression of Fos expression was induced by AP-1 binding activity following focal cerebral ischemia.

343.4


We have previously reported that a 30 s, high +G (head-to-foot internal load) exposure of > +25G causes global cerebral ischemia in rats (Wercchan and Shahed, The Physiologist, 35: S143-146, 1992). Also, there were ++25G exposures have been shown to cause transient brain edema (Shahed et al. Aviat. Space Environ. Med. 63:5, 1992). In the present study HSP70 expression was investigated as an early marker of cellular stress.

Methods: Protocol I: Rats were exposed to +2.5G for either 15, 30 or 60 s in a small animal centrifuge, and brains were collected immediately or 0.5 to 24 hr after the run. Control rats were exposed to +0.5G. Protocol 2: rats were subjected to six 30 s exposures of +22.5G, and brains were collected as in Protocol 1. Total RNA was extracted by RNAzol B method. RT-PCR was performed using 1 μg of RNA for cDNA synthesis and specific HSP 70 primers were used for subsequent amplification.

Results: Expression of HSP 70 did not increase above the control level after 15 or 30 s exposure. Following the 60 s exposure, HSP 70 expression increased at 60 and 180 min after the centrifuge run. In protocol 2, expression of HSP 70 increased at 60 and 180 s after the run.

Discussion: High +G, exposure of at least 60 s duration or six exposures of +22.5G were required to increase expression of HSP70. The time points of increased HSP70 expression were similar to those previously reported for brain edema. This suggests that HSP70 expression could be a suitable marker for monitoring post +G, exposure effects.
**ISCHEMIA: MECHANISMS III**

**439.1**


A well established model of neonatal cerebral hypothyamic ischemia involving subjecting 7 day old rats to unilateral carotid artery occlusion and exposure to 3 hr of hypoxia which results in damage spialateral to the carotid occlusion. In several adult animal models of cerebral ischemia, DNA fragmentation has been noted of apoptosis has been observed. Whether programmed cell death contributes to the damage which occurs following hypoxia-ischemia in neonates is not known.

The presence and identity of DNA fragmentation was investigated in situ by dual-labeling with terminal deoxynucleotidyl transferase and propidium iodide. Breaks in DNA were observed in brain sections ipsilateral to the carotid artery occlusion in rats exposed to 3 hrs of hypoxia-ischemia corresponding to extensive damage observed in the HE&E sections. In some animals subjected to 2 hr of hypoxia-ischemia, the damage was less severe and selective areas of positive DNA fragmentation were observed in the hippocampus, thalamus, caudate nucleus and cerebral cortex. Tissue analysis of these regions confirmed the presence of ladder DNA fragmentation.

This, in model of neonatal hypothyamic ischemia there is evidence that DNA fragmentation indicative of apoptosis can occur suggesting that programmed cell death contributes to the damage observed.

(Supported in part by Heart and Stroke Funds and CanadaFights Fights Stroke Program).

**439.2**

**GENETIC MANIPULATION OF ORGANTOPIC COULTURES WITH AN ADENOVIRUS ANEVOKESS TO CHANGE NEUROANATOMICAL PROJECTIONS.** M. Wiener and E. Angelides.

The structure and organization of thalamocortical (TC) axons in the developing mammalian neocortex is the focus of current investigation. Organotypic cultures of fetal thalamic and neonatal cortical explants permit the in vitro dissection of the signals and mechanisms underlying the development of TC projections. To date, the roles of various molecular and signal transduction molecules is a potentially powerful tool for exploring the development of this circuitry.

Recently, several labs have demonstrated the usefulness of using viral vectors (herpes simplex virus I and adenovirus) to transfect neurons in vivo and in vitro. Replication-defective, recombinant (RDR)-adenoviruses have several advantages over herpes virus vectors, including: (1) high titer, (2) safety and (3) relative long-term expression of the recombinant gene. To explore the possibility for genetic manipulation of organotypic cultures, rat cortical explants, cocultured with thalamic explants, were infected with AdRSV5gal. Infection of cortical explants, with both RDR-adeno and RDR-herpes virus vectors was investigated: (1) labelled thalamic axon growth was also assisted 1-2 weeks post infection. In 2 in vitro experiments where the bilateral lesions were formed between infected and control cortical explants, no gross differences in the extent of thalamic ingrowth or axonal arborization were detected. These data suggest that adenoviruses may be gene vectors for genetic manipulation of target tissue. Future experiments will concentrate on designing recombinant adenovirus for studying target selection and circuitry formation in thalamocortical explants. Supported by NIH grant NS 26733. M. Wi. in NIH postdoctoral fellow (NS 00710).

**439.10**


Apoptosis, or programmed cell death, has been shown to be a mechanism by which cells can die from a cell to commit suicide. CDC is usually associated with intranuclear DNA fragmentation, which may lead to an upregulation of certain proteins such as p53, a member of the heat shock protein family. This study was performed to show that following a unilateral focal stroke, induced in rats by photochemical thrombosis (rose bengal is injected intravenously and the skull is irradiated with a beam of focussed light; Neuroscience 1990, 55, 472; J Neurosurg 1993, 79: 2440) will have a significant role in the area penumbra. We collected brain samples at different times post-stroke and determined immunohistochemically: a) in situ DNA fragmentation by staining the brain slices with anti-digoxigenin antibody to detect digoxigenin labelled-DUTP-DNA adducts (AntiAp; Orion); b) the presence of p53 (antibody PAAb20; Oncogene Science Inc.); and c) the presence of 72 kD heat-shock protein (72 kD antihumononal antibody, Amerham). Following stroke, we observed increases in DNA fragmentation, p53, and 72 kD protein in the cortex ipsilateral to the thomeric core. These increases occurred in different brain regions: DNA fragmentation and p53 increased only in the area penumbra, while 72 kD heat-shock protein increased throughout the cortical cortex, except in the area penumbra. DNA fragmentation was evident as early as 6 hr post-stroke, peaking at 12-14 hrs, and leveling off at 24 hrs. In contrast, p53 and 72 kD immunoreactivity was not increased until 8 hrs and lasted throughout the progressing cortex.

These results suggest that stroke-triggered p53 upregulation might be responsible for the CDC in the area penumbra.

**439.11**

**THE EFFECT OF TRANSIENT GLOBAL ISCHEMIA ON ACTION POTENTIALS, CALCIUM INDUCED CALCIUM TRANSCRETS INS [Ca]I PYRAMIDAL NEURONS. Y. Schiller, C. Sommer, and M. Kissingler.

The effect of global ischemia on the brain's ability to maintain calcium homeostasis and calcium dependent mechanisms was examined. Ischemic conditions were evoked by electrical and synaptic activity in CA1 pyramidal neurons. Brain slices were prepared from mice and sacrificed at 3 days of age. Calcium transients were measured at the soma and at different location along the apical dendrite (10-300um).

The major findings of this study are: 1) The resting [Ca] was not significantly different (control and ischemic) but up to 24 hours after the ischemic event. 48-72 hrs after a small increase of the resting [Ca] was observed. 2) The [Ca] transients evoked by a train of 5 action potentials (synaptic or electrophysically evoked) were not significantly altered up to 24 hours after the ischemic event. 48-72 hrs after the ischemic event, the prolongation of the [Ca] transients was observed. 3) Following a 0.5 second 100Hz synaptic stimulation the peak and decay of the [Ca] transient did not significantly differ in normal and ischemic gerbils 24 hours after the ischemic event. In both groups about 85% of this [Ca] transient was blocked by APV. From these findings we concluded that during the first and potentially reversible period after the ischemic event (24h) no significant abnormalities were detected in the amplitude of synaptic and action potential induced [Ca] transients and in the ability of CA1 pyramidal neurons to eliminate this calcium load.
439.3 LAMINAR SPECIFIC ATTACHMENT AND NEURITE OUTGROWTH OF THALAMIC NEURONS ON CULTURED SLICES OF DEVELOPING CEREBRAL NEOCORTEX. D.E. Essenhigh and A.D. Lander*. Dept. of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

In nervous system development, the growth cones of advancing axons are thought to navigate to their targets by recognizing cell-surface and extracellular matrix molecules that act as specific guidance cues. To identify and map cues that guide the growth of a particular axonal system, the thalamocortical afferents, an assay was devised to examine short-term interactions of dissociated embryonic thalamic cells with living, ~150 μm slices of developing mouse forebrain. Thalamic cells rapidly (<3 hours) and efficiently attached to and extended neurites on pre- and postnatal slices, but a broad zone around the neocortex was generally non-permissive for both thalamic cell attachment and the ingrowth of neurites. This zone coincided with the cortical plate at early stages (embryonic day 13), but later became restricted, in radial-to-cortical fashion, to cortical laminae 2/3. Thus, at each stage, thalamic cells in vitro avoided just that area that thalamic axons confront, but generally do not enter, in vivo. In addition, neurites that extended on some layers were found to be significantly oriented in directions that coincide with the pathways that thalamic axons follow in vivo. Furthermore, these laminar specific behaviors of thalamic cells on cortical tissue were observed when fresh frozen cryostat sections were substituted for living brain slices. The results imply that local adhesive cues and signals that affect process outgrowth are distributed among developing cortical laminae in a manner that could underlie much of the temporal and spatial patterning of thalamocortical innervation.

By investigating what cell-surface and extracellular matrix molecules account for the behavior of thalamic cells in this rapid in vitro assay, we hope to elucidate some of the molecules that control the in vivo development of the thalamocortical pathway.

439.4 CNS-ASSOCIATED CUES ARE REQUIRED FOR CENTRAILY-DIRECTED NAVIGATION BY PERIPHERAL SENSORY NEURONS IN THE EMBRYONIC MURINE NEUROPHYSIOLOGY. L. J. Arices*, K. Johnson, B. C. Johnson, Neuroul Res. Center and Dept. of Physiol. and Biophysics, Unv. of Alabama, Birmingham, AL 35294 & Dept. of Zool. and Genetics, Iowa State Univ., Ames, IA 50011.

The axons of peripheral sensory neurons in Xenopus laevis medially navigate through several potential choice points toward the CNS where they then segregate into discrete axon tracts which can be dissociated by differential expression of surface antigens. The earliest population of sensory cells arise in discrete clusters called sensory and pathway selection by individually dyed-filamented sensorg growth cones is highly specific. We have shown earlier that one potential source of guidance information proximal to the CNS was the outgrowing axons of central pioneer neurons. To examine the potential influence of CNS-associated cues, we performed CNS ablations early on embryonic day 18. Gangliocytic primordia were removed from anesthetized embryos which were allowed to survive for 6-8 days before labeling them with the monoclonal antibody Lea3-2 to stain all sensory axons. Control preparations involved surgically opening the body wall but not removing the gangliocytic primordia. Pathfinding and selective fasciculation in control preparations were indistinguishable from normal. In contrast, in each of 110 hemisegments deprived of CNS (15 animals), the seminal set of axons appeared undirected. In all cases the majority of seminal axons fascicolized extensively upon one another following a tight circular pathway around the neuropile. Normally these axons project some distance from this point. There were also a few instances where normally separate peripheral fascicules became tightly fascicolized along abnormal pathways. These results demonstrate that the CNS normally provides cues necessary for correct navigation, and in its absence the peripheral neurons fail to fasciculate with each other. Further evidence in support of this is that later developing extramurally oriented neurons do differentiate and project axons in the absence of the CNS, but appear unable to identify an appropriate direction for growth. Instead they form short tendrils of axons that do not join their normal pathways. Thus, the peripheral sensory neurons of larvae require CNS-derived gradient information. We are currently further examining the nature of the cues provided by CNS neurons necessary to directafferent innervation. Supported by NSF 9209237 and a Sloan Foundation Fellowship (Jla) and NH 26867 (Jle).


In the embryonic spinal cord, commissural axons pioneer a circumferential pathway from one hemisphere to the floor plate at the ventral midline. Floor plate cells secrete a factor that attracts these axons in vivo, suggesting that the ventral growth of these axons may be directed partly by chemotropism. Floor plate cells have also been shown to secrete a (possibly distinct) diffusible factor that promotes the outgrowth of commissural axons in collagen gels in vitro. Using outgrowth of commissural axons into collagen gels as an assay, we have purified from embryonic spinal cord a protein, netrin-1 and netrin-2 (28 kD and 75 kD, respectively, by SDS-PAGE), that mimics the outgrowth-promoting activity of the floor plate. We have also identified the distinct activity in embryonic brain that potentiates their effects. Cloning of cDNAs encoding the two netrins show that they are homologous proteins (72% identical) which are exclusively expressed in the CNS. We have been able to clone a laminin-related protein required for the circumferential migration of cells and axons in the nematode C. elegans. This homolog suggests that the guidance of growth cones in the vertebrate spinal cord and the nematode are directed by similar molecular cues.


The guidance of axons to their targets in the developing nervous system is believed to involve diffusible chemotrophic factors secreted by target cells. Floor plate cells at the ventral midline of the spinal cord secrete a diffusible factor (or factors) that promotes the outgrowth and fasciculation of commissural axons that recruits these axons in vivo. Two proteins from embryonic brain, netrin-1 and netrin-2, possess the outgrowth-promoting activity of floor plate cells. Here we report that during the period of commissural axon growth to the ventral midline, netrin-1 is expressed at high levels in the floor plate region, whereas netrin-2 is expressed more widely and at lower levels in the ventral spinal cord. Moreover, heterotopic expression of either netrin can mimic both the outgrowth promoting and the long-range chemotrophic activities of floor plate cells. Thus, netrin-1 and netrin-2 are chemotropic factors that may guide commissural axons in the developing spinal cord.


We have been characterizing the expression and function of several genes involved in growth cone guidance at the midline of the Drosophila central nervous system (CNS). In mutations of the commissureless (cmn) gene, growth cones miss the midline instead of staying on their own side (Seeger et al., 1993). In mutations in the roundabout (robo) gene, growth cones that would normally stay on their own side instead now cross the midline. The cmn gene encodes a novel transmembrane protein expressed by a subset of midline cells (Teet, Seeger, and Goodman, submitted). Closing of the rob expression domain gives rise to an embryonic CNS midline defect. Here we describe the characterization of a third gene – D-nefrin – that was cloned on the basis of homology to the two vertebrate netrin genes and the nematode unc-6 gene. In the nematode, UNC-6 appears to control circumferential guidance. In vertebrates, netrin-1 and netrin-2 are expressed by the floor plate and ventral neural tube, respectively, and can function in vitro to attract commissural growth cones. The D-nefrin gene is located on the X chromosome. It is strongly expressed by a subset of midline cells developing, and by visceral mesoderm. Based on its homology to the netrins/UNC-6 and its expression pattern, we have named this gene that D-nefrin plays an important role in midline guidance. To test this model, we are using genetic approaches, including the analysis of specific growth cone loss of function mutations to trace in embryos and to construct constructs which produce specific patterns of ectopic D-nefrin expression.


The Semaphorin genes encode a family of highly related secreted and transmembrane proteins which appear to function during growth cone guidance. Antibody blocking experiments have shown that gangliocytic Semaphorin I (formerly Fasciclin IV) is required for the proper guidance and fasciculation of the T11 growth cones in the limb bud of the grasshopper embryo (Kolodkin et al., 1992). Chick collapsin, a secreted member of the Semaphorin family, is capable of causing growth cone collapse in an in vitro assay (Luo et al., 1993).

We previously identified two members of the Semaphorin gene family in Drosophila (Kolodkin et al., 1993). Semaphorin II (Sema II) is a secreted protein that is structurally similar to cholinergic growth cone repulsive molecule Semaphorin IV, a 500 amino acid Semaphorin domain, a single immunoglobulin domain, and a C-terminal tail. In the embryo, Sem II is expressed by a small subset of cells in the CNS and by muscle 31 in the body wall of the T3 segment, suggesting a role in the generation of neuromuscular connectivity.

We have taken a genetic approach to characterize the function of Sema II. Adult flies homozygous for this mutation have a variety of behavioral phenotypes including partially penetrant flightlessness, visual orienting defects, and abnormal drinking behavior. To gain insight into the origins of these behavioral defects, we have examined the effect of the sema II loss-of-function mutations on the innervation of muscle 31 in the embryo and larvae. To examine this effect, we have used transgenic constructs to ectopically express Sema II by novel sets of muscles. In this way, we hope to determine the function of this secreted Semaphorin during growth cone guidance and target recognition.
439.9 EXPRESSION OF THE SEMAPHORIN III GENE DURING DEVELOPMENT OF THE RETINOGENETIC SYSTEM. E. N. Hems et al., A. I. Kolkodin, C. S. Goodman, and C. J. Shatz, Department of Biology, University of California, Berkeley, CA 94720.

The Semaphorins are a family of highly related secreted and transmembrane proteins that appear to function during growth cone guidance. Semaphorin III (Sema III, also known as Fas IV) in the grasshopper is a transmembrane protein that functions in vivo in growth cone guidance (Kolodkin et al., Neuron, 1992). Sema II in Drosophila is a secreted protein that is essential for adult behavior and survival (Kolodkin et al., Cell, 1993). Chick collagen, a secreted protein that is closely related to D-Sema II, can function in vitro to cause DFG growth cone collapse (Lu et al., Cell, 1993).

Based on the homology of human Semaphorin III (Kolodkin et al., Cell, 1993), we used PCR to clone mouse Sema III which is likely to be the homologue of chick collagen. Here we conduct in situ hybridization using a mouse Sema III cDNA to analyze Sema III expression during mammalian development. Whole-mount embryos (E9.5) and tissue sections (E13.5, E15.5, P7, and P21) were hybridized with antisense and sense riboprobes. Some of the structures showing the most prominent labeling with the M-Sema III probe include posterior diencephalon and branchial arches at E9.5, ventral neural tube motoneurons, cells surrounding dorsal root ganglia, and pial and cortical plate at E13.5, retina and olfactory tract at E15.5, and olfactory tract, cerebellar Purkinje cells, and cortical layers 6b and 5 at P20.

439.11 AXONIN-1 AND Ng-CAM-LIKE IMMUNOREACTIVITY DURING THE DEVELOPMENT OF THE RETINOGENETIC SYSTEM IN THE CAUCAL G. Rager, P. Morino and P. Sonderegger, Institute of Anatomy, University of Freiburg, CH-7100 Freiburg, and 1Institute of Biochemistry, University of Zürich, CH-8057 Zürich, Switzerland.

The development of the retinotectal system seems to follow the rule of chronology (Rager et al., Anat. Embryol. 179, 133-148, 1988; Rager et al., J. Comp. Neurol. 310, 239-250, 1991). To show this type of order, we used immunocytochemical expression of cell adhesion molecules could play an important role. Recently, two axonal adhesion molecules, axonin-1 and Ng-CAM, have been found to interact during the process of axon growth (Sonderegger and Rathjen, J. Cell Biol. 109, 1397-1394, 1990). We have observed the expression of axonin-1 and compared it with the distribution of Ng-CAM in the retina and in the retinotectal system of the chick during the period of development. At stage 18 both axonin-like (A-L) and Ng-CAM-like immunoreactivities (Ng-CAM-LI) are clearly present in the area where first retinal ganglion cells (RGCs) are generated. The immunoreactivity spreads synchronously with the formation of ROGs over the developing retina. From stage 21 on the inner proliferative layer was also stained according to its temporal gradient of maturation. In later stages the outer plexiform layer and the inner segments of photoreceptors show immunoreactivity, too. The development of A-L and Ng-CAM-LI along the optic nerve, chiasm, optic tract, and in the superficial layers of the optic tectum seem to follow the chronotopic distribution of axons as it was found by earlier morphological investigations. Older axons seem to loose their A-L which allows us to localize the position of newly formed axons. The fact that A-L and Ng-CAM-LI go in parallel with the formation and maturation of axons allows us to suggest that axonin-1 and Ng-CAM may play an important role in the organization of the retinotectal system. Supported by Swiss N.S.F. grant 31-34409.2.


Ordely neuronal maps of visual space are created in the fly brain by precise retinal cell (R cell) contacts to the optic lobe. To identify genes that regulate R cell connectivity we screened Drosophila larvae for mutations affecting the R cell projection pattern. We analyzed over 5000 IMS mutagenized lines for aberrant R cell projections and isolated 30 interesting loci. Each mutation was analyzed to determine if the gene underlying the defect 1) is required in the R cells, 2) affects R cell fate determination, 3) affects cell fate determination of the optic lobe. A genetic mosaic analysis was used to determine if the gene is required in the eye. In this procedure a patch of mutant eye tissue is generated in an otherwise normal fly. Those mutations that produce a defect in the R cell projection lobe are likely impaired in the R cells. Neural cell fate determination was assessed in both the eye and the optic lobe. In particular we wanted to eliminate from further analysis those mutations that can be rescued in the eye (i.e., in situ transcription) and those that result in defects that may be a secondary consequence of changes in neuronal identity. Three mutations were isolated that are required in the eye but not in the R cell fate. These mutations represent candidate genes which function in the R cells to generate precise connections with their targets.

439.13 DROSOPHILA RECEPTOR TYROSINE PHOSPHATASES ARE EXPRESSED IN THE DEVELOPING VISUAL SYSTEM C. Desai, E. Popova, B. Hamilton and R. Zinn*, Dept. of Biology, Caltech, Pasadena, CA 91125.

Several receptor-linked protein tyrosine phosphatases (R-PTPs) appear to be expressed on results in adult CNS in Drosophila. We purified such one R-PTP, DPTP69D, using anti-PPase against horseradish peroxidase (HRP) as immunofluorescence antigen. Anti-HRP antibodies recognize a carbohydrate epitope expressed in the optic ganglion and the optic lobe. This RPTP was expressed on HRP reagents that were applied to CNS in the eyes. In third instar larvae, DPTP69D expression is restricted to subsets of neuronal processes in the brain, ventral nerve cord, and eye disc. In particular, DPTP69D is expressed on photoreceptor axons and on the neurites of the developing lamina, medulla and lobula complex. Interestingly, DPTP69D is also expressed in the optic lobe in a similar pattern, but at lower levels. We have made DPTP69D mutants; these are viable with normal embryonic CNS. We are examining the visual system in larvae and adults of these mutants. We are also trying to disrupt DPTP69D by mobilizing a P element located 8 kb downstream from DPTP69D.

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Regulation of DOPAMINE D3 RECEPTORS EXPRESSED IN HEK-293 CELLS: M.A. Piachev, K.D. Burns and P.E. Molloy. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-0894.

We have previously reported a drug-induced increase in the density of D2 receptors expressed in transfected HEK-293 cells (Mol. Pharmacol. 44:371-379). D2 receptors have distinct structural and pharmacological properties. It was of interest to determine whether the D3 receptor regulates in a fashion similar to the D2 receptor. A stable cell line was created by transfecting the D3 receptor, under the control of a cytoplasmic promoter, into human embryonic kidney cells (HEK-293). The density of receptors after treatment with compounds known to be agonists or antagonists at D2 receptors were measured in homogenates using radioligand binding assays with the following compounds: [125I] transf-7-hydroxy-2-PiPAT or the D3 antagonist [125I] NCCQ-298. HEK-293-D3 cells express a single class of D3 receptor per mg of protein. Exposure of HEK-293-D3 cells to quinpirole (5.0 μM) for 18 hr resulted in a 77% increase in the density of binding sites for both [125I] trans-7-hydroxy-2-PiPAT and [125I] NCCQ-298. This increase in density was both time- and concentration-dependent. Exposure of cells to haloperidol (5.0 μM) for 18 hr, resulted in a 57% increase in the density of D3 binding sites. Treatment with the dopamine antagonist haloperidol (5.0 μM) for 18 hr resulted in a 145% increase in the density of D3 receptors. The increase in the number of D3 binding sites induced by haloperidol was also concentration-dependent. The data suggest that both D2 agonists and antagonists increase the density of D3 receptors expressed in transfected HEK-293 cells. (Supported by MH41654, and NS18591.)

440.3 PRODUCTION OF MICE WITH A CONSTITUTIVE DEFICIENCY OF THE BETA-ADRENERGIC RECEPTOR KINASE (AR) AND HOMOLOGOUS RECOMBINATION: B. Gloumann, J. Le Royɛwitz and M.G. Coronel, HUH, Duke University Medical Center, Box 2587, Durham, NC 27710, USA.

Protein phosphorylation is a key event of the transduction process that underlies the activation and desensitization of B/arrestin receptors. A constitutive &BK-1 (-/-) mice was created which allows for the analysis of events that would normally be regulated by receptor desensitization. The consequences of this change in the phosphorylation status of B2-adrenergic receptor kinase (BKAK) were evaluated in the context of B2-adrenergic receptor function. The results of these experiments were compared with that of a wild type control.
NUCLEAR PROTEINS FROM YOUNG AND OLD RAT TISSUES INTERACT DIFFERENTIALLY WITH THE D2 Dopamine RECEPTOR GENE PROMOTER. I.M. Deloam, C.J. Roth, T. Hinova and M.M. Mosandl. Molecular and Genetic Section, Laboratory of Cellular and Molecular Biology, NIA, NIH, Baltimore, MD 21220; Genetic Pharmacology Unit, Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

Loss of dopamine receptors and/or changes in their activity may lead to compromised neuronal functioning in aging and certain neurodegenerative diseases. To study the mechanisms of regulation of the D2 dopamine receptor gene in different tissues with aging, we carried out gel mobility shift experiments using nuclear extracts from young and old rats and DNA fragments or oligonucleotides containing different parts of the D2 promoter. A 415 bp DNA fragment containing sequences from -900 to -76 relative to the major transcriptional start point and a 378 bp fragment containing sequences from -75 to -30 bound to position(s) present in nuclear extracts prepared from rat cortex, cerebellum, hippocampus, striatum, and kidney. In addition, the 378 bp probe bound to nucleofected AF2 promoter as well as promoter(s) present in AF2-enriched extracts. We have also shown that an oligonucleotide containing sequences from -75 to -30 binds protein(s) present in extracts from rat cortex, hippocampus, and liver. In several cases, nuclear factor(s) present in extracts from cortex, cerebellum, hippocampus, or striatum formed DNA-protein complexes with different gel mobilities than those formed with liver extracts, suggesting that the identity of some of the nuclear factors binding to the D2 promoter may differ between brain and peripheral tissues. Furthermore, with equal amounts of total nuclear protein, greater binding was observed using cerebellar and liver extracts from young rats than from old rats. These results suggest that the concentration and/or binding activity of nuclear factors interacting with the D2 dopamine promoter may change with age. Further characterization of putative DNA regulatory sites and regulatory proteins in different issues of young and old animals is in progress.

STEROID RECEPTOR MEDIATED EFFECTS OF NEUROSTEROIDS. R. Ruppert, J. H. M. Reul, T. Trapp, B. van Steenberg, C. Wetzel, W. Ziegglitsbgerger and F. Holbofer.* Max-Planck Institute of Psychiatry, Clinical Institute, Department of Neuroendocrinology, Krappealistr. 2-10, 80804 Munich, FRG.

Several 3α-hydroxy steroids accumulate in the brain after local synthesis or after metabolization of steroids that are provided from the adrenals. As this may occur independently from peripheral sources these steroids are called neurosteroids. The 3α-hydroxy ring A-reduced pregnane steroids allopregnanolone and tetrahydrodeoxycorticosterone are believed to interact with intracellular receptors but enhance γ-amino butyric acid (GABA)-mediated chloride currents. The present study shows that these neurosteroids can regulate gene expression via the progesterone receptor (PR). Although they do not bind to the PR of other species, they confer an exclusively nuclear localization of the PR. However, the induction of DNA-binding and transcriptional activation of the PR requires intracellular oxidation of the neurosteroids into PR-active 3α-pregane steroids. Thus, in physiological conditions, neurosteroids regulate neuronal function through their concurrent influence on transmitter-gated ion channels and gene expression. These findings extend the concept of a 'cross-talk' nuclear hormone effects and provide a new lead for the therapeutic application of these steroids in neurology and psychiatry.

PINEAL α-1D ADRENERGIC RECEPTOR mRNA: HIGH ABUNDANCE, DEVELOPMENT AND ADRENERGIC REGULATION. S.K. McCune*, S.L. Coto, D.E. Brentman and D.C. Klein. Section on Developmental and Molecular Pharmacology and Section on Neuroendocrinology, Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

Adrenergic receptors mediate tissue responses to catecholamines. Binding studies have documented the presence of α-1 adrenergic receptors in the pineal gland and pharmacological studies have demonstrated a role of these receptors in the adrenergic regulation of many aspects of pineal function, including melatonin production. In an effort to better define α-1 adrenergic receptors in the pineal gland, α-1B adrenergic receptor expression was examined in situ hybridization and Northern blot analysis. Developmental expression of the α-1B adrenergic receptor subtype was determined by Northern blot and densitometric analysis of in situ hybridization of brain sections through the pineal as well as sections of individual pineal glands. A-1B receptor mRNA was highest in the early perinatal time periods and decreased with maturation. The abundance of mRNA encoding this receptor subtype in the pineal gland was extremely high as compared to brain and other tissues; a strong hybridization signal was detected on Northern blot with 2 µg of total RNA.

Pineal blots of pineal mRNA from day and night animals showed a 2.5-fold increase in the α-1B adrenergic receptor mRNA at night without a similar increase in the mRNA encoding Gα-PI2. This change is probably due to nocturnal adrenergic stimulation of the pineal gland, because in vitro stimulation of the pineal gland for 4 hours with norepinephrine (1 µM) induced a selective 2-fold increase in the abundance of α-1B adrenergic receptor mRNA.

The presence of high levels of α-1B adrenergic receptor mRNA in the pineal gland and evidence of neural regulation of expression make this tissue an excellent model for further analysis of this receptor subtype.
441.1


Department of Pharmacology and Toxicology and *Anatomy, West Virginia University School of Medicine, Morgantown, W. Va.

CRF is a 41 amino acid peptide first isolated and characterized in 1981 (Vale et al). CRF is part of a dynamic system which regulates central and peripheral components of the stress response (G1 and Nemeroff, 1991). Depolarization-induced, calcium-dependent release of CRF from fetal neural cultures of the amygdala has been previously demonstrated (Crafty and Birley, 1994). Fetal cell neurones (passage 6 of the cell line on glass coverslips (12-10 cells/slip)) yield a 5:1 mixture of neurons and glia after 15-18 h. Immunostaining with anti-neurofilament marker, anti-glial acidic protein and anti-glial fibrillary acidic protein (A-252/66) by CRF and GAD antibodies revealed a colocalization of CRF and GAD in amygdala. Furthermore, immunohistochemical characterization of these cells in culture demonstrates compartmentalization of neurons into clusters connected by a network of fiber bundles. The evidence points towards the amygdala maintained in the in vitro culture system. Supported in part by NSF (IBN-9222263).

441.2


The Scripps Research Institute, Dept. of Neuropharmacology, CA 92037, 441.3

**LEARNING DEFICITS IN TRANSGENIC MICE WITH CENTRAL OVEREXPRESSION OF CORTICOTROPIN-RELEASING FACTOR.** S.C. Hetherich, M.P. Strowig-Forsey, J.M. Gold, E. Battegay, F.E. Bloos, G.F. Koch, W.W. Vale, T. Milm. Phi. The Scripps Research Institute, Dept. of Neuropharmacology, 10666 N. Torrey Pines Rd., La Jolla CA 92037, 1Neurocine Biosciences, Inc., 3505 Science Park Rd., San Diego CA 92121, 2Oregon Health Sciences University, Dept. of Microbiology and Immunology, P.O. Box L220, 3181 Sam Jackson Park Rd., Portland OR 97201 and 3The Salk Institute, Clayton Foundation Laboratories for Peptide Biology, 1010 North Torrey Pines Rd., La Jolla CA 92037.

Transgenic (TG) mice with central overexpression of corticotropin-releasing factor (CRF) exhibited a Corticis Disease-like picture, elevated plasma levels of ACTH and corticosterone, increased behavioral reactivity to stressors and an anxiogenic-like state which is reversed by central administration of a CRF-antagonist. Moreover, centrally administered CRF alters learning/memory processes in animal behavioral models. In this study we have demonstrated that overexpression of CRF in transgenic mice may be associated with memory impairment and anxiety-like behavior. Supported in part by grants DK 26741 to WWV and GKF and by the Kleberg Foundation.

441.3

**PRENATAL STRESS CAUSES HYPERSECRETION OF CORTICOTROPIN-RELEASING FACTOR (CRF) FROM AMYGDALE.** M. Crans*, H. Ward, E. Johnson, A. Azzaro, D. Birley.

Dep'ts of Pharmacology/Toxicology, Behavioral Medicine/Psychiatry, and Neurology, West Virginia University School of Medicine, Morgantown, WV 26506.

The neurohypophyseal, corticotropin-releasing factor (CRF), has been found to be distributed throughout the central nervous system. CRF has a role in emotional and behavioral states including stress and anxiety. The amygdala, a limbic structure important in the delicate control of emotions and autonomic responses to stress, contains CRF nerve terminals and CRF receptors. We have examined CRF release from the amygdala of adult male offspring of dams exposed to daily saline injection (0.1 ml, s.c.) from G14 to G21. This prenatal stress model produces offspring that are hyperresponsive to stress (Ward et al, this volume). In light of the role of amygdala CRF release in anxious responses, we hypothesized that exposure to prenatal stress would increase CRF release from the amygdala. CRF release from amygdala slices (1 mm²) was time-dependent, depolarization-induced and calcium-dependent. There was a 32% increase in depolarization-induced CRF release from the amygdala of prenatally stressed rats. Prenatally stressed offspring also had an increase in the amygdala. The data suggest that CRFergic neurotransmission in amygdala is upregulated after prenatal stress, an effect that may contribute to the hyperresponsive state observed in these animals. Supported in part by NIH (2507RR05433-31 and GM07039) and UHA.

441.4


The central nucleus of the amygdala (CgA) is known to be involved in the regulation of the parsymaptic and parovative coping and stress-related responses to conditioned and acute stressors. Neuroanatomical studies revealed that the majority of the corticotropin-releasing hormone (CRH) containing neurons in the CgA have direct connections with autonomical regulatory nuclei in the hypothalamus. In this study we have used the Roman High Avoidance (SRA/verh) and the Roman Low Avoidance (RLA/verh) rats (kindly provided by P. Duricovic, Zürich) to examine the effect of CRH on the CgA in both a stress-free and a conditioned stress situation. The RHA/verh and RLA/verh rats are considered to use an active and a passive coping strategy respectively. A 7 min infusion of 30 ng CRH (in 0.5 ml aCSF) into the CgA of freely moving male RHA/RLA rats under acute-free conditions, led to an increase in heart rate and behavioural activation only in the RHA treated animals, leaving the RLA rats unaffected. This is in contrast with the conditioned situation in which only the RLA/verh males responded. These results suggest a differential CRH central amygdaloid control of the behavioural and physiological stress response in the two selective strains of rats. Subsequently experiments using CRH in nDNA and Fos immunochemistry are in progress to obtain further experimental evidence for a differential central amygdala CRH modulation in the RHA/RLA rats.

441.5

**CORTICOTROPIN-RELEASING FACTOR INFUSED INTO THE LOCUS COERULEUS INCREASES NORADRENERGIC RELEASE IN MEDIAL PREFRONTAL CORTEX.** Gennady N. Smagin, Artur H. Swiergiel, Glenn Guenther* and Adrian J. Dunn, Dep't of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.

Previous studies have indicated that intracerebroventricular administration of corticotropin-releasing factor (CRF), has been found to be distributed throughout the central nervous system. CRF has a role in emotional and behavioral states including stress and anxiety. The amygdala, a limbic structure important in the delicate control of emotions and autonomic responses to stress, contains CRF nerve terminals and CRF receptors. We have examined CRF release from the amygdala of adult male offspring of dams exposed to daily saline injection (0.1 ml, s.c.) from G14 to G21. This prenatal stress model produces offspring that are hyperresponsive to stress (Ward et al, this volume). In light of the role of amygdala CRF release in anxious responses, we hypothesized that exposure to prenatal stress would increase CRF release from the amygdala. CRF release from amygdala slices (1 mm²) was time-dependent, depolarization-induced and calcium-dependent. There was a 32% increase in depolarization-induced CRF release from the amygdala of prenatally stressed rats. Prenatally stressed offspring also had an increase in the amygdala. The data suggest that CRFergic neurotransmission in amygdala is upregulated after prenatal stress, an effect that may contribute to the hyperresponsive state observed in these animals. Supported in part by NIH (2507RR05433-31 and GM07039) and UHA.
**441.7** SENSITIZATION OF THE EXCITATORY EFFECTS OF VASOPRESSIN OR CORTICOTROPIN-RELEASING FACTOR ON THE ACOSTATIC STARTLE REFLEX BY VASOPRESSIN ADMINISTRATION TO RATS: A COMPUTERIZED APPARATUS. K. H. Hopper, Y. L. Levenson, W. S. Hodes, S. R. Brown, M. Davis. Dept. of Psychiatry, Yale University School of Medicine, 34 Park St., New Haven, CT 06510

In the rat, evidence now suggests a neurotransmitter function for arginine vasopressin (AVP). AVP, in its various autocrine, paracrine, and endocrine forms, can modulate aversively reinforcing stimuli. In this study we used a computerized apparatus to investigate the effects of AVP on the acoustic startle reflex in the rat. AVP (30, 100, 300, 1500 pg ICV) was infused on Day 1. On Day 2, 48 hours later, all animals were injected with a single test dose of AVP (300 pg ICV) and AVP was subcutaneously injected on Day 1. The results showed that 30 pg of AVP on Day 1 did not sensitize the effect of AVP on Day 2, but 300 pg ICV did (p<0.05).

Because stress is known to activate release of both AVP and corticotropin releasing factor (CRF) and ICV CRF is known to increase baseline startle, we wondered if AVP would also sensitize the excitatory effect of CRF on the acoustic startle reflex. To test this, various doses of AVP (3, 30, 300, 3000 pg) were infused ICV on Day 1. On Day 2, 48 hours later, all rats were infused with a dose of CRF (0.25 pg) that usually has no effect on baseline startle. The results showed a non-monotonic excitatory effect of CRF on startle, with the combination of 30 pg AVP/25 pg CRF showing a significant increase (p<0.05) over vehicle/vehicle or vehicle/25 pg CRF. The time course of the effect was similar to that usually seen when 1 μg of CRF is given ICV.

Taked together, these results show that a single infusion of AVP sensitized the excitatory effect of either AVP or CRF on the acoustic startle reflex, when given 48 hours later. This may provide a model system for analyzing how prior stress leads to enhanced reactions to subsequent stressors and dysregulation of the stress response.


The neurohypophyseal peptide oxytocin has been implicated in the mediation of several forms of affiliative behavior. Marked species and gender differences have been found in monogamous and polygamous rodents, suggesting that oxytocin may play a role in species-typical patterns of social behavior. Using in vitro receptor autoradiography with a selective oxytocin receptor ligand ([125]I-Chlg[5]Tyro(Me)2

**441.9** PROXIMAL SEPARATION FROM PUPS REESTABLISHES OXYTOCIN CONTROL OF MATERNAL BEHAVIOR IN EXPERIMENTAL RAT MOTHERS. C. A. Podreka, J.M. John, B. M. Faggin, G. Ayres and J. D. Caldwell. BIDC, Dept. of Psychiatry and Psychoendocrinology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, N.C. 27599.

We previously reported that separating pups from the proximal separation (PS), a condition in which pups were isolated in small metal mesh cages within each dam's cage so that the dam could smell, hear and see but could not touch them, for 6 days beginning on Day 5 of lactation markedly depleted immunoreactive oxytocin (OT) neural cell bodies in many forebrain sites compared to rat dams subjected to total separation (TS) or no separation (NS) from pups. In recent studies, we found that all rats subjected to 4-6 days of PS (10/10) or NS (10/10), but only 4/11 rats subjected to TS, showed the increased OT levels observed in rats that had been given free access to pups (Fisherman's exact probability, p<.01). Injection of OT, an agonist peptide, but not normal saline, into the VTA (a site that receives projections of OT neurons located in the fornix) significantly inhibited the rapid emergence of retrieval and reaching behavior in dams subjected to PS for 4-6 days. In contrast, infusion of OTA into the VTA had no inhibitory effect on the emergence of retrieval and reaching behavior in dams subjected to PS for 4-6 days. Our behavioral pharmacological findings indicate that the increase in OT immunoreactivity that occurred in the rats subjected to PS is due to increased OT release. These observations also suggest that, shortly after parturition and the OT-dependent behavior of the dam, either OT or OT binding sites are necessary for the rapid rise in OT levels that are observed in rats subjected to TS.

**441.10** COLOCALIZATION OF OXYTOCIN (OXT) AND C-FOS IMMUNOREACTIVITY IN FEMALE HAMSTER BRAIN. D.C. Whitman* and H.E. Albers. Lab. of Neuroendocrinol. & Behav., Dept. of Biol., Georgia State Univ., Atlanta, Ga. 30303.

Central administration of OXT facilitates the expression of lordosis in rodents. In addition, central administration of an OXT antagonist reduces or eliminates gonadal hormone-stimulated lordosis. Since OXT appears to mimic the effects of progesterone (P) on lordosis, the present study tested the hypothesis that OXT influences lordosis by mediating the effects of P on lordosis. Double immunocytochemical techniques were used to examine whether OXT neurons also exhibit c-fos immunoreactivity (a marker of cellular activity) in the brains of OVX hamsters treated with estradiol benzoate (EB) (unmated), EB plus P (unmated) and EB plus P (mated). Preliminary analyses indicate that OXT- and c-fos-immunoreactivity were colocalized in the paraventricular and supraoptic nuclei of mated hamsters. The lack of c-fos immunoreactivity was not observed in either of the unmated groups. This study was supported by NSH 92-22099.

**441.11** POLYMORPHISM AND GENETIC MAPPING OF THE HUMAN OXYTOCIN RECEPTOR GENE ON CHROMOSOME 3 J. Hillander, E. C. Urbaneke, R. Peap and D. Goldman. Laboratory of Neurogenetics, National Institute of Health-National Institute on Alcoholism and Alcohol Abuse, Rockville, Maryland

Central administration of oxytocin has been reported to facilitate affiliative and social behaviors, in functional harmony with its well known behavioral effects on milk ejection. The biological effects of oxytocin could be perturbed by mutations occurring in the sequence of the oxytocin receptor gene, and it would be of interest to establish the position of this gene on the human linkage map. Therefore, we identified a polymorphism at the human oxytocin receptor gene. A portion of the 3-translated region containing a 30 bp CA repeat was amplified by polymerase chain reaction (PCR) and evaluated by electrophoresis, revealing a polymorphism with two alleles occurring with frequencies of 0.77 and 0.23 in a sample of Caucasian cephalic parents (n=70). The CA repeat polymorphism we detected was used to map the human oxytocin receptor to chromosome 3p25-3p26, in a region which contains several important gene families, including loci for Von Hippel-Lindau disease (VHL) and renal cell carcinoma.

**441.12** AN ANXIOLYTIC ACTION OF OXYTOCIN IS ENHANCED BY ESTROGEN IN THE MOUSE. M.M. McCarthy* & D. Goldman. Department of Physiology, University of Maryland, Baltimore, MD. and Laboratory of Neurogenetics, NIAAA, Rockville, MD.

The neuropeptide oxytocin exerts broad effects on social and affiliative behaviors and has been speculated to exert an anxiolytic action but this hypothesis has not been rigorously tested. In Experiment 1, NIH-Swiss ovxated mice were pre-treated with 10μg estradiol (E2) in oil or saline 24 hours prior to testing. Ten microliters of a combination of oxytocin significantly increased percent and total time spent on the open arms and the number of entries onto the open arms. The arms were decreased when oxytocin was co-infused with both E2 and E6-treated females. There was no effect on motor activity or head dips in the hole board apparatus in this experiment. Injection of oxytocin in NIH swiss males did not significantly alter behavior. Levels of activity were comparable to E2-treated females. In Experiment 2, NIH-swi-ovx’d females bearing in-dwelling cannula aimed at the lateral ventricle were pre-treated with E2 or saline and infused ICV with either oxytocin or vasopressin (40μg/saline) and behaviorally tested on the day post-inj. The percent and total time spent on open arms was significantly increased by oxytocin regardless of estradiol treatment and the percent and number of entries onto the open arms was increased by the combination of E2+oxytocin over all other groups. The combination of E2+vasopressin significantly decreased the percent time spent on the open arms compared to other groups. This combination in general exerted opposite effects to that of E2+oxytocin. There was an increase in time spent head-down in the hole board apparatus in E2-treated females but not in the vasopressin treated females. These findings indicate that oxytocin can act as an anxiolytic and that E2 enhances that effect. Furthermore, E2 may be exerting opposite effects on vasopressin, causing it to act as an anxiogenic. Brains are prepared for receptor autoradiography and results will be reported.
442.1 ASTROCYTES IN THE DEVELOPING MACAQUE MONKEY RETINA C. Gliond, C. Deuter and M.A. Kirby, Zoology & Neurobiology, Ruhr-University Bochum, 44800 Bochum, Germany. Department of Pediatrics & Div. Perinatal Biology, Loma Linda University, Loma Linda, CA 92350

In mammals, two types of macroglia are known to exist in the retina, Müller cells and astrocytes. While Müller cells are ubiquitous throughout the retina, astrocytes are only found in vascularized retina or vascularized retinal regions. It has recently been shown that there is an exception to this rule: in the paraventral region of the adult monkey retina macaque astrocytes or astrocyte-like cells exist even though this retinal region is heavily vascularized. To test if the paraventral region is astrocyte-free or at all stages of development or if it is secondarily deserted by astrocytes, we investigated the spatial distribution of astrocytes in fetal macaque monkey retinae using GFAP immunocytochemistry. Fetuses were obtained by Cesarian section under aseptic conditions, deeply anesthetized with pentobarbital and perfused through the heart with 3.4% paraformaldehyde. The retinas were dissected free and processed for GFAP immunocytochemistry as wholemounts.

At embryonic day 120 (term gestation is 165 days), i.e. shortly after formation of the foveal pit, a distributional pattern of astrocytes similar to that found in adults is evident. Astrocytes are found at all retinal eccentricsities with the exception of the paraventral region. Fetal astrocytes are small with delicate processes, but already adherent in their association with axon bundles and blood vessels and their segregation into two subpopulations, one to be found in the ganglion cell layer, the other in the nerve fiber layer. These data together with preliminary observations in even younger foeti (90D) suggest that the paraventral region of the monkey retina is avoided by astrocytes prior to the time of foveal pit formation.

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442.2 CHANGES IN RPTP-ß EXPRESSION DURING GLIAL CELL DIFFERENTIATION. P.D. Canoll, S. Petracecka, G. Barnes, J. Schiliesseringer L.M. Murphy and J.R. Green, Dept. of Pharmacology, N.Y.U. Medical Center, New York, NY 10016.

RPTP-ß is a receptor type tyrosine phosphatase that is expressed in glial and neuronal progenitors (Canoll et al., Dev. Brain Res. 75:293, 1993). Nucleotide probes to RPTP-ß were hybridized to three major transcripts on northern blot analysis. Two of the transcripts (9.5 kb and 7.0 kb) encode transmembrane glycoproteins which contain a carbonic anhydrase related domain, distinct extracellular portion and tandem phosphatase domains in the intracellular portion. The third transcript (8.4 kb) encodes a secreted chondroitin sulfate proteoglycan which contains the RPTP domain, but does not contain the intracellular phosphatase domains (Maurel et al. PNAS 91:2512, 1994). In situ hybridization and northern analysis show that the relative abundance of the 3 transcripts changes during brain development. During postnatal development, the 9.5 and 6.4 kb transcripts (transmembrane forms) are predominantly expressed in glial progenitors located in the preretinal subventricular zone. A similar transition in expression can be induced by treating glial cells with the differentiating agent dibutyryl cAMP. The different forms of RPTP-ß have very different structure, and most likely perform different functions. If this is true then changes seen during glial cell differentiation may relate to important changes in the role glial cells play during brain development and regeneration.

442.3 SCHWANN CELL EXPRESSION OF CD9 IS REGULATED BY NERVE CRUSH AND SUBSEQUENT REGENERATION. S.A. Banerjee* and P.H. Patterson, Division of Biology, Caltech, Pasadena, CA 91125.

CD9 is a 26 kd cell surface glycoprotein with four transmembrane domains that has been implicated in signalling in platelet activation, and is present in both the peripheral and central nervous systems (PNS and CNS). In the PNS, CD9 is expressed in several neuronal populations including the Schwann cells of the autonomic nerve. CD9 expression follows a developmental time-course in the nerve that closely parallels that of the myelin genes P0 and myelin basic protein. Here we report the response of CD9 following injury to the nerve. CD9 and P0 mRNA levels were analyzed following nerve crush in 30-day old rats. CD9 mRNA drops approximately 10-fold in the nerve distal to the crush, similar to the change in P0 mRNA. By two weeks following injury, when axons are known to be rapidly regenerating, CD9 and P0 mRNAs recover to levels slightly higher than those found in the normal adult. By 30 days, levels reach those found in the normal adult. A smaller, but transient response is also observed in the part of the nerve proximal to the crush.

These results suggest that Schwann cell expression of CD9, like P0 expression, requires the presence of axons. This would also be consistent with our observation that when primary Schwann cells are placed in culture, CD9 immunoreactivity is lost. We are currently investigating the role of axons on CD9 expression in cultured Schwann cells.

442.4 Human Fetal Spinal Cord Slice Cultures: A Model for the Study of Neurodevelopment and Neuropathology. W.E. Greer*, K.M. Weidenheim and W.D. Lyman, Department of Pathology, Albert Einstein College of Medicine, Bronx, New York, 19461.

Human fetal spinal cords are obtained from abortuses that range in age from 20 to 24 weeks of gestation. Spinal cords are stripped of meninges and cut into 80mm transverse sections. The slices are cultured on collagen-coated tissue culture inserts in chemically-defined media. Slices have been cultured for up to 28 days under these conditions. Histological and immunocytochemical examination of the cultures reveal the presence of neurons, oligodendrocytes, microglia, astrocytes, ependyma and endothelial cells. Indirect immunofluorescent confocal microscopy shows intact myelin basic protein positive sheaths around neurally-differentiated positive axons. These myelinated axons can be observed by summation of optical sections and span over 60µm. Additional evidence of active myelination during the culture period is observed by light and electron microscopy. Some axons contain mitochondria with distinct cristae and are surrounded by large inner loops of a myelinating oligodendrocyte process. Inner loops are found inside layers of compacted myelin.

After one week in culture, the slices are surrounded by an outgrowth of cells. The outgrowth area contains microglia, astrocytes, and oligodendrocytes as determined by morphology and immunocytochemical labeling. This culture system provides a unique model to study the development of the human central nervous system and neuropathologic changes that result from exposure to inflammatory cytokines, infectious agents such as HIV, drugs of abuse, or physical damage.

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442.5 REGULATION OF TRANSFORMING GROWTH FACTOR ALPHA (TGFα) GENE EXPRESSION IN HYPOTHALAMIC ASTROCYTES BY METABOTROPIC GLUTAMATE RECEPTORS. Y.L. Ma*, F. Baze and S.R. Ogawa. Division of Neuroscience, Oregon Regional Primate Res. Ctr., Beaverton, OR 97006.

Excitatory amino acid (EAA) neurotransmitters influence the initiation of mammalian puberty by stimulating the secretory activity of neurons producing luteinizing hormone releasing hormone (LHRH). Evidence also exists that TGFα, a trophic factor of glial origin, contributes to this action via a paracrine mechanism involving glial production of eicosanoids. The functional relationships that may exist between these two regulatory systems are not known. Since glial cells are endowed with both ionotropic and metabotropic glutamate receptors, we have initiated experiments to determine if glutamate expression of the TGFα gene is subject to EAA-mediated regulatory inputs. Purified astrocytes from neonatal rat hypothalamus were exposed to glutamate receptor agonists including glutamate itself, N-methyl-D-aspartate (NMDA) and the metabotropic receptor agonists quisqualate and ACDP (aminoacyclopentane-1S,3R-dicarboxylic acid) (100µM each). Changes in TGFα mRNA levels were measured by quantitative reverse transcription-polymerase chain reaction at different intervals after initiation of the treatment (15 min 24h). The results showed that both glutamate and metabotropic receptor agonists, but not NMDA, rapidly upregulated TGFα gene expression (within 15 min exposure), with maximal mRNA values being reached between 8-16h. These initial findings suggest that hypothalamic astrocytes are targets for EAA acting via metabotropic receptors, and that EAA may represent one of the neuronal signals involved in the control of glutamate expression of the TGFα gene during development. Supported by NIH Grants HD25123, HD18185 and RR0163).


Acellular basal lamina (BL) allografts are known to exhibit reduced immunogenicity. It is also known, that the extent of host axonal regeneration through acellular BL grafts is reduced in comparison to cellular grafts. In the present immunogenicity study, isogenic (genetically identical) and allogeneic (genetically different) Schwann cell populated acellular BL grafts were transplanted into host rats to define their immunogenicity. Inbred strains of Fischer (FR) and Buffalo (BF) rats were used. Acellular BL grafts were pre-treated, by finite-diameter FR rat nerve. Acellular BL nerve were placed in dishes with cultured Schwann cells established from FR or BF rat naves. After 7 days, 2 cm long Schwann cells axonal regeneration and remodeling was created gap in the FR host peroneal nerve. Transplanted nerves were analyzed at 1, 2, 4 and 8 weeks later to determine their immunological fate and axonal regeneration through them with light microscopy. Acellular BL grafts populated with isogenic FR Schwann cells survived and were supportive of host axonal regeneration through them. On the other hand, acellular BL grafts with allogeneic BF Schwann cells underwent rejection and were unsuccessful. The results indicate that cultured Schwann cells continue to exhibit immunogenicity. In this study, it is indicated that isogenic Schwann cells to populate acellular BL grafts in attempts to enhance their axonal growth supporting function. (Supported by NIH Grant NS-24834.)
442.9 MICROGLIAL REACTIONS TO FOCAL RETINAL INJURY IN THE RABBIT. M.E. Humphries* and S. Moore, WA Retinas Pigmentosa Research Centre, Lions Eye Institute, Perth, Western Australia 6009.

As preliminary experiments to examine the role of microglia in retinal injury reactions, the microglia were labelled using nucleoside diphosphatase (NDPase) histochemistry following argon laser photocoagulation lesions in the peripheral retina in rabbits. At 2, 6, 24 and 48h, and 7, 14 and 21 days after photocoagulation, retinal lesions were made using a 100mW argon laser (200μm diameter) in pigmented rabbits, animals were anaesthetised, perfused and the retinas immersion fixed in 4% paraformaldehyde containing 8% sucrose and 5% DMEM. The next day the retinas were isolated and incubated at 37°C in cytidine diphosphate as a substrate, wholemounted and analysed. Normal retinas had microglia in the nerve fiber layer (NFL) the inner plexiform layer (IPL) and outer plexiform layer (OPL). The distributions were similar to those previously reported (Schnitzer, J. Comp. Neurol., 282, 1990, 779). Following lesioning the IPL microglia had processes pointing into the lesion by 2hrs and had migrated to leave a vacant zone around the lesion by 6hrs, this vacant zone was gradually filled by 7 days. The NFL microglia did not react until 7 days when they became aligned with degenerating axons between the lesion and optic disc. By 14 days these cells were no longer reactive but reactive cells were now found extending from the lesion to the periphery and by 21 days the reaction was largely over. The OPL contained increased numbers of microglia from 2hrs after lesion until 14 days but by 21 days the levels were reduced again. Thus, the microglia in each layer of the retina reacted in different ways reflecting the injury response of the other cells in each layer.

442.10 INTRACELLULAR CALCIUM REGULATION IN ASTROCYTES IN ADULT RAT SPINAL CORD PREPARATIONS. O. Theriault**, L.R. Mills*, University of Toronto Hospital and Playfair Neuroscience Unit, University of Toronto, Toronto, Ontario, Canada. M5B 2S.

We have previously described the presence of a metabolotropic glutamate receptor subtype (mGluR1α) on a subpopulation of astrocytes in the rat spinal cord (Theriault et al., 1993; Nature). The subpial location of these astrocytes is correlated with the characteristic region of tissue survival which follows traumatic spinal cord injuries (Theriault, 1993), and our electrophysiological studies demonstrate a neuroprotective effect of mGluR1 activation followed in vivo and in vitro (long-term culture; Theriault et al., in prep.). To determine the mechanisms underlying the neuroprotection, we have developed an in vitro preparation of the spinal cord. Adult female Wistar rats in anaesthetised, a thoracic laminectomy performed and a 200μm by 400μm strip of the dorsal columns was removed. The isolated strip was secured in a chamber and incubated in 95% oxygen-5% carbon dioxide for 100 minutes, using confocal microscopy we have examined changes in intracellular calcium ([Ca²⁺]i) levels of spinal cord astrocytes in response to kainate, and trans-ACPD. Real-time fluorescence in spinal cord astrocytes were increased two-fold following application of 100 μM kainate, and five-fold after ionophore. Interestingly, preliminary experiments with 100 μM trans-ACPD in uninjured cords revealed detectable changes in [Ca²⁺]i levels. Ongoing studies are examining [Ca²⁺]i regulation in clip compression-injured spinal cord preparations. Theriault et al. (93) 2nd Int. Neurotrauma Symp. July 4-9, Glasgow, Scotland; Theriault and Hampson, (1996) Soc. Neurosci. Abstr. 22, 748; Theriault (1995) 5th Int. Symp. Nerv. Regen.; Dec. 8-12, Ascona Conf. Cen. for P.

443.2 HOMOSYNAPTIC LTP AND PTP OF SENSORMOTOR SYNAPSES MEDIATING THE TAIL WITHDRAWAL REFLEX IN APlysia ARE REDUCED BY POSTSYNAPTIC CALCIUM-DEPENDENT HYPERPOLARIZATION. M. Curt and L.T. Wallace, Dept. of Physiology & Cell Biology, University of Texas Medical School at Houston, TX, 77225.

Tetanization of nociceptive tail sensory neurons (TNS) causes LTP of monosynaptic connections to tail motor neurons (TMNs) lasting >90 min in isolated ganglia (Walters & Byrne, JNeurosci 5:662, 1985). It was not known if this potentiation was homosynaptic or heterosynaptically mediated, but Lin & Glanzman (ProcSocLond, 255:113, 1994) recently showed clear homosynaptic LTP of sensomotor synapses in dissociated cell preparations. LTP in culture was blocked significantly by homosynaptic hyperpolarization during tetanization. As a first step in determining mechanisms and functions of homosynaptic LTP in defensive circuits, we have examined the dependence of TMN-TMN potentiation on TMN membrane potential in whole ganglia. After 2 baseline tests, half the TMNs were tetanized (10 x 0.4 ms trains, 25 Hz) each with the TMN at normal resting potential. The other half of TMNs were over-potentiated, that is, both tetanized and hyperpolarized, by the same amount, except that the TMN was hyperpolarized to -120 mV during the tetanus. EPSPs were then tested at 5 min intervals for at least 25 min afterwards. As shown in the graph, LTP in the ganglia was significantly reduced by TMN hyperpolarization during the tetanus (120% of baseline vs 70% of baseline at 25 min; t=6.77, p<0.05). Interestingly, LTP observed 1 min after tetanization was also reduced by TMN hyperpolarization (157% vs 24%, t=3.04 p<0.05). Observations of homosynaptic LTP in nociceptor synapses of Aplysia and rat (Randic et al., JNeurosci 13:S228, 1993) suggest that LTP (perhaps NMDA receptor-mediated) might be a primitive mechanism for storing memory of injury.
443.3
TEMPORAL SPECIFICITY OF HEBBN LONG-TERM POTENTIATION OF APLYSIA SENSORIMOTOR SYNECTES IN CELL CULTURE. X. Y. Lin and D. L. Glanzman*. Dept. of Physiological Sci., UCLA, Los Angeles, CA 90024.

Long-term potentiation (LTP) of in vitro Aplysia sensorimotor synapses can be induced by pairing brief stimulation of the presynaptic sensory neuron with strong depolarization of the postsynaptic motor neuron (Lin & Glanzman, Proc. R. Soc. Lond., B 255:943-950, 1993). This pattern of stimulation, which occurs during classical conditioning of the Aplysia withdrawal reflex (Carew et al., 1981, 1983), specifically presentation of the CS (weak stimulation of the siphon) and US (strong shock) yields brief sensory neuron activation together with strong motor neuron depolarization (Frost et al., 1988). Classical conditioning of the withdrawal reflex might therefore be mediated in part by Hebbian potentiation of sensorimotor synapses. However, Lin and Glanzman found that weak sensorimotor and postsynaptic stimulation to induce Hebbian LTP, whereas during behavioral conditioning the onset of the CS occurred 0.5 sec before the onset of the US.

We have systematically varied the interval between sensory neuron stimulation and motor neuron depolarization to investigate whether the induction of Hebbian LTP of in vitro sensorimotor synapses exhibits temporal specificity. The interval between pre- and postsynaptic stimulation was varied from -3.0 sec (the onset of motor neuron depolarization preceded the onset of sensory neuron stimulation by 5.0 sec) to + 5.0 sec (the onset of sensory neuron stimulation preceded the onset of motor neuron depolarization by 5.0 sec). We find that an intersensitive interval of +0.5 sec produces significant LTP of sensorimotor synapses. But the temporal specificity of Hebbian LTP for these synapses does not match that reported for the CS-US interval in conditioning of the reflex (Hawkins et al., 1986). Therefore, although Hebbian modulation of sensorimotor synapses may contribute to classical conditioning (see Murphy & Glanzman, 1994), other cellular mechanisms must also be involved.

443.5

Persistent activation of the CAM-dependent protein kinase (PKA), which occurs during the development of long-term presynaptic facilitation of the sensory-to-motor neuron synapses is required for the defensive reflex, results from proteolytic activation of regulatory (R) subunits. Persistence of kinase activity, which requires new protein synthesis, lasts for at least 24 hr. Hegde et al. (PNAS 90:4746) presented evidence that this protein synthesis is mediated by the APT-ubiquitin-proteasome pathway. Using a variety of subcellular fractionation and immunoechemical techniques, we find that both ubiquitin and the proteasome complex are abundant in all parts of the neurons: cell body, proximal and distal terminals. We predict that the degradation of R subunits is regulated by induction of some new proteins. Consistent with this idea, we find that an immediate early gene induction by PKA encodes a 25,000-molecular-weight protein with similarity to the L-type vertebrate ubiquitin C-terminal hydrolases (UCH). Peptide antibodies against the predicted sequence encoded by the Aplysia mRNA recognize an M, 29,000 protein, which, like vertebrate UCH, is expressed only in nervous tissue. Low molecular-weight UCHs are believed to process ubiquitin precursors to monoubiquitin. Nonetheless, we find this Aplysia UCH immunoreactivity to be associated with proteinase. Eytan et al. (JBC 268:1668) showed that high molecular-weight UCHs are associated with proteasomes and postulated that the ubiquitin chains cleaved from degraded proteins clog the complex, thereby slowing degradation. It is attractive to think that the low molecular-weight UCH (which we also find to be associated with proteasome in rat brain) also can enhance degradation. We are also looking for the induction of other proteins that might regulate subunit degradation during the development of long-term memory.

443.6
POSTSYNAPTIC MODIFICATIONS IN LONG-TERM FACILITATION IN APLYSIA: UP-REGULATION OF EXCITATORY AMINO ACID RESPONSES. L-E. Tradescant* and V.F. Castellucci. Lab. of Neurobiology and Behavior, ICRM, Univ. de Montreal, Quebec, H2W 1R7.

Long-term sensitization of the gill and siphon withdrawal in Aplysia is accomplished by facilitation of sensory-motor synaptic connections in the abdominal ganglion which depends on new protein synthesis. This phenomenon has been previously shown to involve presynaptic growth and an increase in transmitter release without any change in the size of miniature EPSPS. At the postsynaptic level, a reorganization of the motoneuron to the sensory terminal would be required to parallel the formation of new synaptic contacts. We show here that 24 hr after an application of S-HIT which produces long-term synaptic facilitation (LTF), the receptor L30 is immobilized to the postsynaptic complex. But acid agonist of the synaptic receptors is increased (day2/day1 = +64% +/- 11.9%, n=9). Inhibition of protein synthesis in the whole ganglion using anisomycin or limited to the postsynaptic neuron by injection of kainin, a ribosome-inactivating toxin, blocks this enhancement (+12.5 +/- 19.2; n=5) and -21.5 +/- 4.4%; n=6). The postsynaptic inhibition of protein synthesis however fails to block 24 hr LTF. These results show that long-term facilitation is independent of postsynaptic protein synthesis, the data are still compatible with a model of LTF that involves coordinate pre- and postsynaptic changes. The latter alterations may be initially independent of protein synthesis, but may gradually become dependent on transcription for stages of LTF lasting more than 24 hr. An increase in the number of functional postsynaptic receptors in a reserve pool may also prime the postsynaptic neuron for subsequent learning-associated plasticity. Funded by MRC of CANADA (MT-12099).

443.7

The synaptic growth that accompanies S-HIT-induced long-term facilitation of the defensive reflex is mediated by post- and pre-synaptic changes associated with cell culture. S-HIT is associated with a down-regulation of cell adhesion molecules (CAMs) on the surface membrane of the sensory neuron (Mayford et al., 1992). Down-regulation may be related to the activation of the extracellular pathway leading to internalization and apparent degradation of APcAM (Bailey et al., 1992). Which of the two types of isoforms is internalized, the transmembrane (TM) or the GPI-linked (GPI)-linked? To address this question, we have selectively expressed epitope-tagged constructs of the two isoforms in cultured sensory neurons. By combining thin section EM with gold-conjugated APcAM antibodies, S-HIT leads to a decrease in antibody labeling intensity (51 % vs. 2.2 ± 0.7% ± 0.011). By contrast, S-HIT has no effect on either the surface distribution (26±8μm ± 1.8 vs. 25.6 ± 2.4, n=6) or internalization (1.7 ± 0.5 vs. 2.2 ± 0.9) of the GPI linked form. These results show that S-HIT internalizes the TM form of APcAM, but not GPI-linked constructs. The TM form highlights the potential regulatory significance of its intracellular domain, which contains a PI3K motif (thought to target degradation) and has two conserved leucine-rich repeats (Kanai and Kandel, 1994). The availability of TM constructs with deletions of, or mutations in, the cytoplasmic tail should now allow us to determine which part of this module triggers internalization and which part targets degradation.

443.8
TAIL SHOCK DIFFERENTIALLY MODULATES TWO FORMS OF SYNAPTIC PLASTICITY IN INHIBITORY INTERNEURON L30 OF APLYSIA. T.M. Fischer* and T.J. Carew*. Yale University, Departments of Psychology and Biology, New Haven, CT 06520.

In the siphon withdrawal reflex of Aplysia, activation of the L30 inhibitory interneurons, either directly or by mild tail stimulation, produces significant long-term depression (LTD) and post-tetanic potentiation (PTP) of the L30 IPSP (Fischer and Carew 1993a,b). In the present study we describe the suppression of LTD in L30 by a modulatory stimulus, tail shock.

Siphon transmission L30 was measured as follows: (1) IPSPs were recorded in L30 following a baseline measurement; (2) S-HIT was directly applied to the isolated ganglia to reveal any post-synaptic currents (IPSPs) in L29 excitatory interneurons (voltage clamped at -85 mV). Following a baseline measurement, L30 was directly exposed to an S-HIT pulse (5 sec at 12 Hz) to potentiate the synapse. Two forms of L30 activity-induced plasticity were examined: (1) Frequency facilitation (FF), the enhancement of the L30 IPSP during intracellular activation at frequencies greater than last S and IPSPS in the train; and (2) post-tetanic potentiation (PTP), the additional enhancement of the IPSP following activation (measured 20 sec after the train). Prior to tail shock, the largest increase in the L30 IPSP occurred 20 sec following activation; PTP was three times greater than FF (P<0.05; 8). 50 sec following tail shock, the baseline IPSP was significantly reduced (P<0.05; P<0.05; also see Fred et al., 1988), yet the L30 IPSP was still significantly larger (P<0.05; compared to baseline) than the IPSPs observed before tail shock. PTP values were significantly different between FF and PTP (P<0.02). However, no significant enhancement of the IPSP was observed following activation: there was no difference between FF and IPSP (P=0.41). Tail shock thus appears to selectively suppress one form of plasticity, potentiation of the L30 IPSP following activation (PTP), while sparing another form (FF). Additional data indicate that tail-shock suppression of PTP may last at least 40 min. We are currently examining the cellular mechanisms of this suppression, as well as its functional consequences (see Blais et al., this volume).
443.9 

**HYPOSOMATIC DEPRESSION IN TAIL SENSORY NEURONS IS NOT THE MECHANISM OF HABITUATION OF TAIL-INDUCED TAIL OR SIPHON WITHDRAWAL IN APLYSIA. M. Stoecker, and T.J. Carew. Departments of Psychology and Biology, Yale University, New Haven, CT. 06520**

Hypoosomatic depression of sensory neuron (SN) output is considered a primary cellular event in several systems. We have examined the contribution of hypoosomatic depression in tail SNs to habituation of tail siphon withdrawal. An Aplysia tail siphon withdrawal was both direct and indirect SN input to tail motor neurons (MNs); and (2) siphon withdrawal, which has only indirect SN input to siphon MNs. In behavioral experiments, we used tail stimulation (ST) to significantly habituated both tail and siphon reflexes (p<0.01 in each case). In parallel cellular experiments, we examined progressively fewer action potentials in both tail and siphon MNs (p<0.01 in each case).

When tail SNs were repeatedly activated (SNs ISS) with intracellular pulses, significant hypoosomatic depression of the monosynaptic EPSP onto tail MNs was exhibited (p<0.01); depression did not generalize to other, non-activated SNs contralateral to the tail. Hypoosomatic depression of the same SN ISS was examined during behavioral training that caused significant reflex habituation (p=0.01); SNs activated by tail stimulation showed significantly facilitated output to tail MNs (R EPSP increase = 463%, p<0.05). SNs not activated by tail stimulation also showed significantly facilitated EPSPs (R EPSP increase = 365%, p<0.05), indicating that SN facilitation was heterosynaptic.

In summary, by using behaviorally relevant stimuli and simultaneously measuring reflex behavior, MN output, and SN output we found that, while the behavioral responses and MN output significantly decreased during habituation, SN output significantly increased. We are currently exploring whether (1) increased SN output onto inhibitory interneurons, and (2) increased inhibition and/or hypoosomatic depression at interneuronal sites contribute to habituation in these reflex systems.

443.10

**DISTINCT COMPONENT OF PRESYNAPTIC CALCIUM CURRENT IS INCREASED BY CYCLIC AMP AT APLYSIA SENSORIOMOTOR SYNAPSES IN CULTURE. M. Klein*, Lab. of Neurobiology & Behavior, Clinical Research Inst. of Montreal & Univ. of Montreal, Montreal, Quebec H3W 1R7, Canada.**

Synaptic augmentation of transmitter release by serotonin (5HT) at Aplysia sensoriornitor synapses can be mimicked by activation of adenyl cyclase and is blocked by 5HT receptor antagonists. We have previously identified in Aplysia sensory neurons a slowly-inactivating current that is enhanced by 5HT and is cyclic AMP-sensitive. We examined an Aplysia synaptic component that is rapidly-inactivating current that is reduced by FMRFamide and of the two, only the rapidly-inactivating component appears to participate in transmitter release caused by single action potentials under most conditions. Chlorophenyl-cyclic AMP augments synaptic currents at sensoriornitor synapses in culture and also causes an increase in a slowly-inactivating calcium current in the sensoriornitor neurons. However, this latter current differs from the major current modulated by 5HT in that it is not affected by dihydroxyphenylamine antagonists, and, unlike the current described earlier, is not blocked by H7, an inhibitor of protein kinase C in these neurons. The current modulated by the cyclic AMP analog is also not affected by FMRFamide. These findings thus raise the question of whether an increase in a cyclic-AMP-sensitive calcium current may contribute to the enhancement of transmitter release that occurs during synaptic augmentation by 5HT.

Supported by NIMH, NSERC, and a Sloan fellowship.

443.11

**LIKE LEARNED BASIS IN APLYSIA HEAD-VOIDING BEHAVIOR DUE TO ASSOCIATIVE OR NONASSOCIATIVE MECHANISMS. J. Chey, P.D. St. John, M. P. Gudino*, R. Wood. Boston University, Dept. of Cognitive and Neural Systems, Boston, MA 02215.**

A long term behavioral memory for the head-voiding behavior of Aplysia can be induced using flashed lights as an aversive stimulus (Cook and Carew, 1986, 1989). Coupling onset of the lights with a particular direction of head movement results in habituation away from that direction. This bias has been interpreted as a form of operant conditioning, and has been studied with a neural network model based on associative synaptic facilitation (Raymond et al., 1993). We have simulated a recurrent grid-like nonlinear dynamical neural model previously used to explain various data including oscillatory behavior in biological pacemakers. In our model two recurrent neural networks are independent of each other to generate oscillations, which drive the side-to-side head-voiding. Within each channel the frequency and amplitude of oscillations depend on transmitter mobilization dynamics, which exhibit both short- and long-term adaptation. We assume that light onset is increased in an onstate nonspecific to both sides of the diopli. Repeated pairing of onstate increments with activation of one side (the "painted" side) of the diopli caused a head-voiding behavior that is directionally dependent. This model provides a parsimonious explanation of the observed behavior, and it avoids some of the unexpected results obtained with the Raymond et al. model. In addition, our model makes predictions concerning the rate of onset and extinction of the biases, and suggests new lines of experimentation to test the nature of the head-voiding behavior. Supported in part by ARPA ONR N00014-92-J-4015, ONR N00014-91-J-4100, ONR N00014-92-J-1109, Sloan Foundation BR-3123, AFOSR FA9550-92-0449.
In an attempt to identify brain regions in which glutamate receptors may mediate memory impairments, we examined the effects of local application of a glutamate antagonist on the performance of pig-tailed macaques in a delayed nonmatch-to-sample (DNMS) task. Kynurenate, an antagonist at NMDA and non-NMDA glutamate receptors, was microinjected into various areas within the hippocampus, parahippocampal cortex and adjacent fields. We examined the effects of the drug on the performance of monkey trained to perform delayed nonmatch-to-sample with delayed response (DNMS) task. In this study, we have shown that the injection of kynurenate into the hippocampus, parahippocampal cortex and adjacent fields produced memory impairments, which were more pronounced in tasks that required spatial memory than in those that required temporal memory. These findings suggest that the glutamatergic system is involved in the processing of spatial information.
444.10 INTACT ARTIFICIAL GRAMMAR LEARNING IN PATIENTS WITH HUNTINGTON’S DISEASE: B.J. Knowlton, L.R. Squire, & N. Butters, V.A. Med. Ctr., San Diego, CA and Deps. of Psychiatry and Neuroscience, UCSD, La Jolla, CA 92039.

Artificial grammar learning appears to be an example of implicit learning. It is normal in amnestic patients and occurs independently of the medial temporal lobe memory system. Patients with Huntington’s disease (HD), who have striatal damage, are impaired in learning certain motor and perceptuomotor skills which are an example of implicit learning. We tested whether artificial grammar learning depends on the same kind of skill learning by testing patients with HD and 12 control subjects. During the study phase, subjects were shown 23 letter strings generated by a finite-state rule system. Each letter string was presented for 9 sec, and the subjects attempted to reproduce the item. The set of training items was then removed one item at a time, and the subjects were told that the training items were generated by a set of rules, and that their task would be to decide for a new set of items whether or not each adhered to these rules. This classification test consisted of 23 new grammatical items and 23 non-grammatical items. Performance by the patients on the classification test was not significantly different from the performance of the control subjects (69.3±2.6 vs. 84.3±5.6 correct). The patients were marginally impaired when asked to recognize letter strings that had been presented during the study phase (65.4±1.1 vs. 73.3±2.9 correct g=1). Artificial grammar learning appears to be unaffected by the striatal pathology and dementia associated with HD.

444.11 MEMORY CONSOLIDATION AND THE MEDIAL TEMPORAL LOBE: A SIMPLE NETWORK MODEL. F. Alvarez* and L.R. Squire. V.A. Medical Center, San Diego, CA 92161, Dyte, of Psychiatry and Neurosciences, UCSD, La Jolla, CA 92039, and Computer Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

Decade memory has been shown to depend on a system of medial temporal lobe structures that includes the hippocampus and the adjacent cortical areas (entorhinal, perirhinal and parahippocampal cortex). The role of this system is not fully understood, however, as shown by the fact that after damage to the medial temporal lobe, recent memories are impaired but remote memories are preserved. It has been suggested that the medial temporal lobe memory system is involved in a process of consolidation: memories that are initially dependent on this system gradually become established elsewhere. Previous network proposals for how interactions between the medial temporal lobe and neocortex may mediate consolidation have been presented, but they are often not explicit on many key points. We have attempted to synthesize a number of the existing ideas into a concrete proposal that can be implemented as a neural network model. We propose that a key role of the medial temporal lobe in memory is to bind together the different portions of a memory representation that are distributed over separate areas of neocortex. This binding is mediated by fast changes in the connections between medial temporal lobe and neocortex, and within the medial temporal lobe. The medial temporal lobe then directs the slower changes in the connections between neocortical areas that underlie consolidation. We show that a simple neural network model based on this proposal behaves in a way consistent with experimental observation. This model addresses several current questions about consolidation, and provides a reference point for further experimental investigation and a starting point for improved models.

444.12 SHORT TERM MEMORY FOR DURATION IN HYPOXIC SUBJECTS. R.D. Hopkins* and R.P. Kesner, Physiology, University of Utah, Salt Lake City 84112.

Previous research has demonstrated that an hypoxic episode can cause damage to the hippocampus as determined by volumetric MRI analysis. There are several ways in which temporal information can be represented in the brain, including temporal order and duration. Previous research has shown that hypoxic subjects are impaired for memory for novel linguistic and spatial temporal order information. Subjects who experienced an hypoxic episode and matched control subjects (N=9) were tested for short term memory for duration (STMD). The STMD task assessed memory for duration across a variety of delays. Subjects were presented with a single object (square, circle, etc.) on a computer screen, for a duration of 1 or 3 seconds. Subjects were instructed to remember the duration of presentation of the object. After a delay of 1, 4, 8, 12, or 20 seconds, the same object reappeared for 1 or 3 seconds and the subject detected if the object appeared for the same or a different duration. There were 48 trials which allowed for 8 observations for each interval. Hypoxic subjects were impaired relative to control subjects in short term memory for duration. In order to determine if the deficits were due to impaired memory for the items, instead of duration, a control task was developed. The same subjects were presented with a single object for a duration of 1 to 3 seconds and were asked to remember the object. After a delay of 1, 4, 8, 12, 16 or 20 seconds, either the identical object or a different object appeared on the screen. The subjects were asked to determine if it was the same object or a different object. No significant differences between hypoxic and control subjects were found on this task. These results suggest that hypoxic subjects are impaired for memory for duration information, but not for memory for single objects. Hypoxic subjects, with damage to the hippocampus, appear to be impaired in the temporal coding of information.

DEGENERATIVE DISEASE: OTHER I

445.1 TRINUCLEOTIDE REPEAT EXPANSION AND DRPLA (SMITH’S DISEASE): MOLECULAR CHARACTERIZATION OF ATROPHIN-1. R.L. Margolis*, S.H. Li, G.A. Ross, Laboratory of Molecular Neurobiology, Johns Hopkins University, School of Medicine, 720 Rutland Ave, Ross 615, Baltimore, MD 21205.

Smith’s disease (also known as dentatorubral pallidolysian atrophy or DRPLA) is a rare, progressive, fatal neurodegenerative disorder similar to Huntington’s disease. Smith’s disease is characterized by ataxia, choreathetosis, myoclonic epilepsy, dementia, and the genetic phenomenon of anticipation. A few cases have been misdiagnosed as ataxia with onset. Neuropathological findings include prominent cell loss in the dentate nucleus of the cerebellum, the globus pallidus, the red nucleus, and the subthalamic nucleus. An expansion of a CAG trinucleotide repeat encoding polyglutamine in a gene originally identified in our laboratory (CTG·879, Li et al, Genomics, 1993) has now been identified as the putative disease allele (Nagafuchi et al and Koido et al, Nature Genetics, 1994). Northern analysis indicates that the gene, which we have termed atrophin-1, is widely expressed as a 5 kb mRNA in human tissue. Translational sequencing of cDNA from healthy and mutant alleles indicates a transition of a alpha helix to beta pleated sheet. The mechanism by which this transition occurs and is subsequently propagated is unknown. We previously described two subtypes of prion diseases that share a single mutation, Asp to Asn, at codon 178 of the PrP, but are differentiated by a common Met/Val polymorphism at codon 129. We created transfectant cell lines expressing either normal PrP or PrP with the 178Met mutation to determine if the processing of the resulting protein was altered. We used a homologous system, the human PrP coding sequence and the human neuroblastoma cell line M17, to simplify analysis. Our findings show that the 178Met mutation results in an alteration of the metabolism of the prion protein. This work was supported by NIH grant AG-08992 and AG-08155.
Degenerative Disease: Other I


The fundamental event underlying scrapie infection seems to be a conformational change in the prion protein (PrP). Because changes in the conformation of PrP might require the participation of molecular chaperones, we examined the expression of heat shock proteins (Hsp) that are known to function as molecular chaperones in scrapie infected cells. Neither heat shock nor exposure to sodium arsenite induced Hsp synthesis in scrapie infected neuroblastoma (Scn2a) cells in contrast to uninfected, control neuroblastoma (N2a) cells. Constitutively expressed Hsp 73 was observed primarily within distinct, localized areas in the cytoplasm of uninfected cells, upon heat shock it dispersed throughout the cytoplasm and failed to be redistributed into the nucleus/microsomes. Hsp 73 was widely dispersed throughout the cytoplasm and was translocated into the nucleus/microsomes after heat in the N2a cells as observed for other cell types. While most of Hsp 73 could be removed following nonionic detergent extraction of the N2a cells, significant amounts of Hsp 73 remained within a detergent insoluble fraction of the Scn2a cells. These alterations in the stress response observed for the Scn2a cells may feature not only in the pathogenesis of the prion diseases but also in the formation of PrP*.


The prion protein (PrP) is a glycosylphosphatidylinositol (GPI) anchored membrane protein of unknown function. An abnormal PrP isoform, partially resistant to proteases, is a hallmark of prion diseases in humans and animals. Immunoblot analysis of normal human brains using antibodies specific to various regions of PrP has revealed the presence of truncated PrP forms. These fragments are not the product of autoxidation, since they were also present in neuroblastoma cells in culture. The most abundant of these fragments is N-terminal truncated and its size is comparable to that of full length PrP. This truncated form is also attached to the cell membrane through a GPI anchor, since it can be released from neuroblastoma cells in culture as well as from brain microsomal fractions by treatment with PIPCL. Our ongoing studies indicate that the relative amount and type of these truncated forms vary according to severity and type of pathology in brains from subjects with human prion diseases, such as Creutzfeld-Jakob disease and Fatal Familial Insomnia. Studies of these fragments may help understand the role of PrP processing in normal and pathological conditions. Supported by NIH grants AG-08155 and AG-08992.

445.5 IS THE APOLIPROPOTEIN E (APOE) Ɛ4 ALLELE OVERREPRESENTED IN DIVERSE NEURODEGENERATIVE DISORDERS (NDD)? J. A. Schneider, G. M. S., M. M.,* Dept. of Pathology and Laboratory Medicine, VA Med. Center & Emory University School of Medicine, Atlanta, GA 30322.

The allele of APOE overrepresented in late onset familial and sporadic Alzheimer's disease (AD) and other amyloid-associated disorders, e.g. Creutzfeldt-Jakob disease and amyloid polyneuropathy, have failed to demonstrate the same for Alzheimer's disease (Kalaria et al. 1993;49:170). We sought evidence of increased representation of the ε4 allele in ND that, like AD, are associated with increased age, neuronal loss, and cytoskeletal pathology. DNA was extracted from paraffin blocks or frozen tissue, and APOE genotype was determined using a polymerase chain reaction/resistase enzyme methodology. The presence of NDE progression to prion encephalopathy (PSP; n=17), Pick's disease (n=6), corticobasal ganglionic degeneration (CBGD; n=7), Parkinson's disease (PD; n=7), diffuse Lewy body disease (DLBD; n=1), multi-system degenerations (MSD; n=7), lobar atrophy without Pick body (n=2), and entorinal sclerosis (n=1). Coexistent AD pathology of varying severity was present in 16 of 48 ND cases. We found at least one APOE ɛ4 allele in 27 of 48 cases of ND (56%) as compared with estimates of 20-25% in the general population and 60-80% in AD (72% in our own series). When the 16 cases with coexisting AD were excluded, 15 of the remaining 32 ND cases (47%) had at least one APOE ɛ4 allele. The proportion of cases with the ɛ4 allele increased to 56%, when only those ND with neuronal cytoskeletal abnormalities or inclusions (PSG, Pick's disease, PD, DLBD, and CBGD; n=25) but without AD were analyzed. In the seven ND cases without concurrent AD or neuronal inclusions, however, an ɛ4 allele was found in only one case (14%). These findings suggest that the APOE ɛ4 allele is overrepresented in ND other than AD, particularly those with neuronal cytoskeletal abnormalities. Further genetic analysis of additional cases is needed to clarify the role of APOE in these diverse disorders. Supported by AG10310 and VA Merit Award.


Quantitative analysis of senile plaque (SP) number, size, and amyloid burden (percent of cortex covered by Aβ) in Alzheimer disease (AD) show that the amount of Aβ deposited correlates with APOE genotype, with APOE ɛ4 > APOE ɛ3 > APOE ɛ3/0. To test the hypotheses that this genotype effect is due either to differences in platelet-derived growth factor (PDGF) or in the kinetics of SP growth and dissolution, we used computerized image analysis techniques to analyze the size distribution of about 400 SP/case in the superior temporal gyrus of 42 AD cases. We discovered a remarkable homogeneity of size distribution that fits a log-normal curve. This size distribution profile suggests that SP growth is not dependent on time or simple surface area, but instead that the interior of SP may be available for Aβ deposition and resolution. Comparing the A8 measures among AD patients with different genotypes, we find increased total Aβ deposition with APOE ɛ4 but no difference in the size distribution curves among genotypes. This suggests that the major difference accounting for the larger amount of Aβ in APOE ɛ4 is formation and stabilization of SP nucleation rather than alterations in ongoing deposition of Aβ. Supported by NIH AG08487.


Increased risk of Alzheimer's disease has been associated with inheritance of different alleles of the apolipoprotein E (apoE) gene (apo4 > apo3 > apo2). The apoE isoforms differ by single amino acid changes in the receptor binding domain: apoE2 has two Cys, apoE3 one Cys, and apoE4 none. ApoE binds Aβ in vitro and immunostains senile plaques (SP). We previously found that an apoE receptor, the low density lipoprotein (LDL) receptor-related protein (LRP) also stains SP, and we proposed that apoE acts as a transport mechanism for Aβ. We have now studied apoE and LRP in CSF. Two new results are evident: 1) LRP is present in CSF, as a novel shed form of the receptor. This form appears to be extracellular, 2) the extracellular ligand binding domain but not the intracellular internalization domain of LRP. Second, in addition to being present as monomers, apoE2 > apoE3 exist as large complexes due to disulfide bonds forming homodimers and apoE4-β heterodimers; ApoE4 does not form these complexes. Because dimers may bind receptors less well than monomers, this difference may be of biological importance.


Copper zinc superoxide dismutase (SOD1) is involved in neutralizing free radicals within cells and has recently been shown to be defective in some familial ALS cases. In this study, we have analyzed SOD1 in sporadic ALS with activity assays and immunocytochemistry. We found no difference in extracellular or intracellular activity between ALS cases or controls. Spinal cord sections from 6 ALS cases, 1 primary lateral sclerosis (PLS) case and 1 control case were stained using 3 different antibodies to SOD1: since astrocytes are involved in neutralizing free radical damage, antibodies to glial fibrillary acidic protein (GFAP) and vimentin were used as independent markers of astrocytes. Our three principal findings from localizations are: 1) ALS spinal cord displayed a reduction or absence of SOD1-reactive astrocytes, compared to the control and PLS cases, and 2) examination of GFAP-stained sections showed that the reactive astrocytes were in the lateral tracts and ventral white and gray matters in ALS and PLS cases, there was a close relationship between astrocytic processes and motor neuron soma. In the control case, which we stained in the A54310 cases and confirmed with morphometry. 3) Vimentin-positive astrocytes were found either in the lateral tracts or in the ventral white matter or in both, in all ALS cases; the case of PLS had none. We conclude that an absence of detectable SOD1 in subpopulations of spinal cord astrocytes and alterations of the motor neuron-glial relationship may play an important role in ALS.

Free radicals have been implicated in several neurodegenerative disorders, such as Alzheimer's disease, Huntington's disease, and more recently amyotrophic lateral sclerosis (ALS). Reduced activity of superoxide dismutase (SOD), an enzyme that protects the cell against free radical oxidative damage, has been detected in serum of ALS patients. We have determined the levels of erythrocyte SOD activity in 29 ALS patients, 20 healthy controls and 13 normal controls by a spectrophotometric assay. We found that erythrocyte SOD activity was significantly reduced (P<0.05; p=0.041) in both SALS (mean ± SEM, 934.88 ± 61.54 U/mg protein, 19%) and FALS (329.88 ± 0.00 U/mg protein, 49%) patients compared to controls (1615.7 ± 70.02 U/mg protein). Values of SOD activity measured in both controls and FALS are closely clustered around their respective means. In contrast, in SALS the distribution of SOD activity seemed to be in two clusters: one in the range of normal activities (n=18, 1139.66 ± 55.33 U/mg protein) and a second (n=11, 599.80 ± 55.33 U/mg protein) close to FALS values. Although, additional investigation is required to clarify the underlying mechanism responsible for the diminished distribution of SOD activity in ALS as well as for the reduction in SOD activity in some of these patients, our data support the hypothesis that free radicals may play a role in the sporadic form of the disease.

Supported by Grants from the Muscular Dystrophy Association and NINDS (1-K08-NS01724-01).


Huntington disease (HD) is a genetic neurodegeneration that preferentially affects the striatum. Patients with Huntington's disease appear hypometabolic with large caloric requirements, atrophy of body fat and muscle. We have searched for a neurochemical evidence consistent with a generalized metabolic defect.

H nuclear resonance spectroscopy demonstrated that persons with clinical Huntington's disease have elevated lactate levels in striatum and occipital cortex. Persons with HD are asymptomatic but genetically positive have normal lactate levels in the occipital al cortex; but lactate may be elevated in striatum. Photic stimulation caused a 3 fold increase in occipital lactate in controls but a decrease of occipital lactate (50% decrease) in HD. 12/14 HD pts treated with abequoline demonstrated a lowering of occipital lactate (29% decrease). In some, treatment with riboflavin/nicotinamide also lowered brain lactate levels. Blood lactate or pyruvate levels were normal. CSF lactate/pyruvate ratios were mildly elevated (12.67±0.38 HD vs 15.82±1.93 controls). Ratios of resting organic/inorganic phosphate measured by MRS in calf muscle are decreased in HD affected (6.5±0.14 HD vs. 9.8±1.4 control).

Many of these abnormalities occur in patients with known mitochondrial disorders. We hypothesized that elevated lactate may be a measure of a pathologic metabolic strain leading to neuronal death and pharmacologic strategies which lower lactate might slow the neurodegeneration.
446.5 PREFERENTIAL EXPRESSION OF MEFC2 IN INHIBITORY INTERNEURONS IN HUMAN CEREBRAL CORTEX. D. Leifer* and K. Wehr. Dept. of Neurology, Yale University School of Medicine, New Haven, CT. MEFC2 (erythrocyte-specific enhancer binding factor 2C) belongs to the MADS family of transcription factors, is expressed in muscle and brain, and activates transcription by binding to the MEFC2 element, a DNA regulatory element essential in certain genes expressed in muscle and brain (Leifer et al., PNAS 1993; 90:1546-50). MEFC2 is present at high levels in the cerebral cortex where it is found in post-mitotic neurons that have migrated to the cortical plate. We have now studied MEFC2 in human brain samples resected during surgery for intractable epilepsy. Strongly MEFC2-immunoreactive (IR) nuclei occur predominantly in layers II, IV, and VI, and are also scattered in layers III and V, whereas more numerous weakly reactive nuclei are found. Some MEFC2-IR nuclei are present in layer I. Many of the strongly MEFC2-IR cells that predominate in layers II, IV, and VI are also strongly MEFC2-IR cells in layers III and V and are also labeled with antibodies against parvalbumin or calbindin, calcium-binding proteins found in GABAergic inhibitory interneurons. These cells have a variety of non-pyramidal morphologies. Many of the weakly MEFC2-IR nuclei in layers III and V are in pyramidal cells, as determined by double labeling with SMI-32, a monoclonal antibody against nonphosphorylated neurofilaments found in pyramidal cells. These results indicate that the level of MEFC2 immunoreactivity correlates with neuronal size and that strong immunoreactivity is present in inhibitory interneurons. The level of MEFC2 expression may therefore have a role in regulating genes that control neuronal phenotype.

446.7 CLONING AND CHARACTERIZATION OF RAT PBX1, A HOMEO GENE DEVELOPMENTALLY REGULATED IN THE CNS. M. Morinobu*, S. Hockfield and L. Redmond, Section of Neurobiology and Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510 In CNS development a homogeneous neuroepithelium gives rise to a multitude of cell types as the result of cell proliferation, differentiation and migration. The regulation of these processes is controlled by many genes whose expression is both temporally and spatially restricted during development. This specificity of expression is presumed to result from the expression of transcription factors. Recently a novel group of human homeobox genes, PBX1, 2 and 3, have been identified. They show a high level of divergence from previously reported homodomain, and only 36% identity to yeast MAEl. Expression of PBX genes was analyzed using an antibody library in searching an embryonic CNS at embryonic day 18 (22.5h) brain (Monica, et al., 1991, Mol. Cell. Biol. 11:6499-6517). To characterize the expression of PBX1 in the mammalian CNS and to investigate its putative role in nervous system development, the rat homologue of PBX1 was cloned by PCR amplification from an adult rat brain library using synthetic oligonucleotides. The clone obtained was used in Northern and in situ hybridization analysis of embryonic and postnatal rat brains. Both studies revealed expression of rat PBX1 in the CNS over the course of embryonic and postnatal development. Rat PBX1 expression is observed in the CNS at embryonic day 15 (E15), peaks in expression postnatally, and is subsequently down regulated in the adult. Rat PBX1 is detected in the cortex at all ages and in the olfactory bulb postnatally. Several structures in the midbrain, diencephalon and cortex show transient expression during development. The temporal and spatial pattern of expression of rat PBX1 mRNA suggests a role for this homodomain protein in mammalian brain development. (Supported by NS28807 to SH)

446.8 MOLECULAR CLONING OF CHICK BRAIN-3.0 AND THE DISTRI BUTION OF ITS RNA DURING DEVELOPMENT OF THE CHICK NERVOUS SYSTEM. Jonas Lindberg, Ioke Williams, Anders Håckström*, Finn Hallbäck and Ted Ebenhald, Department of Developmental Biology. Box 587. Uppsala University, S-751 23 Uppsala, Sweden. Brn-3, a member of the POU transcription factor family, is mainly expressed at high levels in sensory neurons and retina. We wanted to isolate the chicken homologue of rat Brn-3, investigate its mRNA expression pattern development and to examine possible correlation between Brn-3 mRNA and the expression of the neurotrophin high-affinity Trk receptors. Using degenerate oligonucleotide primers and PCR we isolated the chicken Brn-3.0 from spinal ganglia cDNA. This fragment was then used to screen cDNA libraries and a chicken genomic clone in order to get the full length sequence promoter and gene structure. In situ hybridization histochemistry was performed on consecutive sections using probes specific for the detection of mRNA for Brn-3.0 and for the neurotrophin high-affinity receptors. Brn-3.0 was expressed within neurons of many of the cranial ganglia, specifically localized to neurons that project to the spinal cord. In the spinal ganglia expression is strongest in the dorsal root ganglia. Immunoreactivity for the Trk receptor was predominantly found in sensory neurons derived from the spinal cord. Brn-3.0 expression was mainly localized in sensory neurons derived from the spinal cord. The expression of Trk mRNA appears to decrease with age, no decrease in the expression of Brn-3.0 was observed. At the earliest stage examined (stage 11) Brn-3.0 mRNA expression was quite strong in the presumptive neuroblasts migrating from the trigeminal placode, whereas the expression of trkC mRNA was just beginning. This indicates a possible influence of Brn-3.0 on trkC expression in trigeminal placode derived neuroblasts.

446.9 THE REGULATION OF NESTIN EXPRESSION. R. McKay*, T. Hayes, R. Jin*, K. John, O. Brustle, T. Hazl, C. Vicario, S. Josephson, and S. Hockfield. *Yale University School of Medicine, New Haven, MD 20822; #Physiology & Biophys., Hahnemann U., Philadelphia. Expression of the intermediate filament protein nestin is characteristic of stem cells in the mammalian CNS. This conclusion is based on extensive in vivo analysis of the expression of both the endogenous protein and the identification of a tissue specific enhancer in the nestin gene. Nestin is down-regulated in primary cultures of hippocampal cells that differentiate to express neuronal markers. Nestin is also expressed in glial precursor cells. In the adult brain, re-expression of nestin has been observed in reactive astrocytes and in neuroepithelial tumors. We are currently focusing on the identification of regulatory sequences controlling nestin expression. Both gel shift analysis and the generation of transgenic mice are being used to determine whether the same DNA elements are regulating transitions in nestin expression in different cell types. The identification of these regulatory elements in the nestin gene will establish whether the expression of nestin in different cell types reflects the action of common mechanisms.

446.10 RESTRICTED CLONAL ALLOCATION IN THE CHIMERIC MOUSE BRAIN. C. Ruan1, E. Elliott2, R. Leveson3, R. Fawell4, and P. Rakic1. 1Sect. Neurobiol., 2Sect. Immunobiol., 3Dept. Cell Biol., Yale School of Medicine, New Haven, CT 06511 Lineage analysis in Drosophila wing development revealed restricted clonal allocation and led to the concept of compartmentalization. Whether similar principles apply for embryogenesis of the vertebrate nervous system remains an issue under debate. Here we address this issue by examining distribution of clonally related cells in chimeric mouse brains. Stable transduced embryonic stem (ES) cells were generated to express lacZ gene driven by chick beta actin promoter as lineage marker. Mouse brains were generated by implanting ES cells that express with such lacZ(+) ES cells, transferred to foster mother mice, bred to term and sacrificed at postnatal day 10 for examination by X gal histochemical staining. Although X-gal positive ES cells were intermingled with X-gal negative cells in the chimeric brain, the overall distribution was not random but highly regionalized. Each animal exhibited a distinct subset of X-gal(+) cells, and within each colonized structure the density of marked cells could be highly enriched in specific regions. Furthermore, a bilateral symmetry in distribution and density of X-gal(+) cells was a prevailing feature in all examined animals. Thus, for example, when a group of X-gal(+) cells were located in the CA2 region of hippocampus or in a subcortical nucleus, a mirror image distribution was also observed on the opposite side. The data together, the regionalized and bilaterally symmetric distribution of clonally related cells in chimeric mice suggests a restricted and precise clonal allocation exists in the vertebrate nervous system.
EMX2 IS SELECTIVELY EXPRESSED IN GERMINAL NEUROEPITHELIUM OF DEVELOPING DORSAL TELENCEPHALON OF THE MOUSE. M. Giuffrida1,2, V. Broccoli1 and E. Bosco11. 1Dib, Scientific Institute H San Raffaele, Via Olgettina 60, 20132 Milano and 2Istituto di Biologia Generale, Universita di Catania, 95124 Catania, ITALY

We recently cloned two vertebrate homeobox genes, Emx1 and Emx2, related to empty spiracles, a gene controlling very anterior body regions during Drosophila embryogenesis. Both vertebrate genes are expressed in the brain of mouse embryos. They are expressed in the embryonic cerebral cortex in a developmental period, day 10 and day 16 post coitum, corresponding to major events in cortical neurogenesis. In its full extension, days E12.5 to E13.5, their expression domain comprises cortical regions including promordia of neopallium, hippocampus and parahippocampal archipallium. In particular, Emx1 expression seems restricted to cortical regions, mainly in the olfactory cortex. Emx2 expression is also observed in some embryonic neuroepithelial planes includingolfactory placodes and olfactory epithelia. We have now studied in detail their expression pattern in relation to cortical neurogenesis in E12-E16 mouse embryos. Preliminary data show that Emx2 is expressed in germinal neuroepithelium but not in the cortical plate nor in the so-called transition field. Conversely, Emx1 is expressed in both the neuroepithelium and the transition field.

Our previous studies showed that in explant co-cultures, retinal axons from embryonic tectum, embroyonic retina, and posterior tectal slices prepared from animals of all ages, including adult, however, retinal axons reveal different patterns of growth when challenged with tectal axons from animals of different ages. In embryonic tectum, embroyonic retina were almost exclusively very little branching; whereas in postnatal and adult tectum, significantly more ramifications are observed. In order to further characterize the growth of embryonic retinal axons in targets of different age, we have used time-lapse video microscopy to examine the morphology of retinal growth cones as they encounter and begin to invade target tissue. Explants of retinas and tectum were maintained in serum free medium for 15-72 hr. Crystals of DII were placed in the retina 10 hr prior to the beginning of observations. Images of labeled retinal axons were recorded on a low-light video camera every 2 min for a period of 5-8 hr. In general, fibers from E14 retina growing into E14 tectum have simple growth cones which form swellings at the tip of the axons and have few filopodia. Significantly more growth cones were seen extending along other labeled axons than in the older tissue. When retinal explants were co-cultured with postnatal or adult tectum, the growth cones were typically larger (2-5 times those in embryonic tectum), more lamelated, and had longer, more numerous filopodia. Over the 8-hr observation period, some filopodia were observed to stabilize and become collaterals on trunk axons once the growth cone moved ahead. Our results suggest that the interactions between axons and growth cones are affected by the axon-target interactions and the spatial configuration of the target tissue.

(Support: NIH grants T32-MH15761, EY00504, EY00126, EY02621)

44.4 CELL LINEs GENERATED FROM AVIAN EMBRYONIC RETINA BY ONCOCONE-TRANSFERENCE EXHIBIT MATURE GANGLION CELL CHARACTERISTICS AND RESPONSE TO POSITIONAL CUES OF TECTAL MEMBRANES IN VITRO. G. E. Potterberg,* C. Kuechel, B. Eichhorst, M. Zenke, H. Beug & J.U. Schwartz, Dept. of Developmental Biology, D-72076 Tübingen, Germany

We generated cell lines by transfecting quail embryo retina with a retroviral construct containing v-myc into the hormone-binding domain of the estrogen receptor, allowing for the activation of the chimeric protein by estradiol. The oncogene myc was chosen because of its proliferation-inducing capacity and differentiation compatibility. The induction was performed by cocultivation of virus-producing, proliferation-incompetent fibroblasts with avian embryonic day 3.5 quail eyes. In these organ-cultured retinae, cells proliferate and differentiate within a time span of less than 20 hours, a prerequisite for both the generation of stable as well as neurally differentiated cell lines. The transected neuroneuropilues were aspartated into nasal and temporal parts, dissociated, and kept as single-cell cultures. Individual cell colonies were isolated and expanded; these cell lines have been maintained in culture over 6 months with a doubling time of approximately 20 hours and can therefore be considered immortal.

Spontaneously differentiating cells were observed within the cell line cultures, exhibiting morphological features of mature retinal neurons in vitro. Some of these differentiated cell lines displayed the morphological characteristics and marker expression of mature retina ganglion cells. The differentiation rate could be substantially increased by the addition of retinol acid on basic fibroblast growth factors. These differentiated cells maintained functional properties of ganglion cells as revealed by a substrate choice assay. When confronted with substrate stripes of membrane vesicles prepared from target and non-target parts of the tectum, the axonal processes of temporal lines preferred to grow on target stripes, indicating that the cell lines will be useful for the investigation of mechanisms underlying axon-target interactions.

44.5 GROWTH PATTERNS OF CHICK RETINAL AXONS IN RETINA-TECTUM COCULTURES. Stefan Meyer,* Friedrich Rumberler, Max-Planck-Institut für Entwicklungsbiologie, Spemannstrasse 35L, 72076 Tübingen, Germany.

During retinotectal map formation in the chick, growth of temporal axons is restricted to the anterior half of the tectum, whereas nasal axons invade the posterior tectum (Nakamura & O'Leary, 1989). Inspection of a temporal zone is achieved by sidebranch formation and subsequent elimination of overshooting parts of the axon. It is assumed that a repulsive guidance molecule (RGM) is expressed predominantly in the posterior tectum prevents growth of temporal axons into this region (Walter et al., 1987).

In order to investigate axonal growth and branching in vitro, we developed a co-culture assay of retinas with tectum. DII-labeled pieces of retina were cultured together with a piece of anterior and posterior tectum on opposite sides in a collagen matrix. Growth patterns of labeled axons were investigated after 48 hours or continuously by using time lapse video microscopy. As in vivo, temporal axons did not invade anterior tectum, but grew and branched in anterior tissue. Nasal axons grew into and branched in both tissues. These results indicate that failure of temporal axons to invade the posterior tectum is linked to repulsive molecules and not due to attraction by the anterior tectum. When cGMP-gated retinal cells were cultured with the enzyme PI-PLC, temporal axons could invade posterior tissue. This is consistent with the earlier findings by Mee & Cipri. In a different paradigm, both tectal pieces were placed on one side of the explant. Thus, axons first grew through anterior tissue before they reached posterior tissue. We found that axons grew to the anterior tectal piece, whereas nasal axons continued to grow into posterior tissue. Time lapse analysis revealed that temporal growth cones collapsed after they reached the A-P border. We are presently investigating whether such encounters with repulsive tissue can also lead to the formation of new sidebranches at a distance from the growth cone.

44.6 MOLECULAR CHARACTERIZATION OF REGGIE-ANTIGEN: A GROWTH ASSOCIATED CELL SURFACE PROTEIN IN THE RETINOTECTAL SYSTEM OF GOLDFISH T. Schuh,* P.Lottspeich* and C.A.O. Stuermer* Faculty of Biology, University of Konstanz, & MPI for Biochemistry, Munich, Germany. Retina-Antigen, which could be blocked by the monoclonal antibody M802, was identified as a cell surface protein appearing on goldfish retinal ganglion cells (RGCs) during the first 8 hours after optic nerve transection (ONS) (Paischle and Stuermer, Soc. Neurosci. Abstr. 1991). In addition to its expression on RGCs and regenerating RGC axons, Reggie antigen is also found on cells lining blood vessels in the CNS and on microglial cells in the injured optic nerve. Reggie-Antigen was purified by immunoadsorption chromatography with M802 from total membrane fractions of adult goldfish brains and from total larval goldfish (age: 30-60 d). In SDS PAGE the immunospecific protein has an apparent molecular weight of 48 kD from total membrane fractions of adult goldfish brains and from total larval goldfish (age: 30-60 d). We have cloned a complete cDNA fragment coding for part of the Reggie-Antigen. It was obtained by PCR using degenerate oligonucleotide primers derived from internal peptide sequences of the immunospecific protein. So far the CDNAs clone and the peptide sequences show no homology to known proteins. Anchored PCR strategy as well as CDNAs library screening is now being performed to obtain a full length clone.


We studied, after placing a crystal of DII (fluorescent marker) in an experimental eye, the retinogeniculate pathway at 0, 3, 7 and 15 days of postnatal development in the C57BL/6 mouse. We used controls and animals which were macroscopically and microscopically naive or deafferented at birth. Results show that, in the controls, the map of visual projections in the dorsolateral geniculate nucleus is present at D7. Deafferentation causes the loss of this map and an increase in ipsilateral visual projections. Until D7, the retinofugal fibres present many varicosities which thereafter start to regress and at D15 become more mature. These varicosities, which are observed on animals treated with a free radical scavenger (EGB761, IEPSEN). It seems that at D7, the varicosities on the fibres are slightly more numerous ; the same for the pericellular collateral fibres of the superficial optic tract.


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447.9

TYROZINE PHOSPHORYLATION AT FILIPODIAL TIPS OF VERTEbrate GROWTH CONES


We have previously reported that phosphorylation is concentrated at filopodial tips of Aplysia growth cones, suggesting a role in regulating filopodial function. We have now used immunochemistry and phosphorylation studies at filopodial tips of the growth cones of several types of cultured vertebrate neurons: neonatal rat hippocampal neurons, embryonic chick retinal and sympathetic ganglion neurons, and retinal ganglion cells. Accumulation of phosphorylation at filopodial tips was sensitive to the culture substrate. Increasing the density of laminin on the substrate increased neurite outgrowth from mouse retinal ganglion cells, but decreased the percentage of filopodia displaying strong tip phosphorylation. Concentration of phosphoryryosine at filopodial tips and its sensitivity to the substrate suggest the potential importance of phosphorylation in pathfinding decisions.

To identify proteins associated with phosphoryryosine in filopodial tips, we double-stained growth cones with antibodies to phosphor-tyrosine and each of several proteins known to associate with actin filaments and to be substrates for tyrosine phosphorylation. Contactin, integrin and ezrin (or a component) frequently co-localized with phosphor-tyrosine. Tensin, vinculin and Paxillin were at tips and associated proteins found at focal contacts, were observed in lamellipodia around cell bodies, but not in growth cones.

447.11


The establishment of precise retinal connectivity during development is essential for the retina to transmit visual information. The factors that regulate convergence and divergence during development between bipolar, amacrine, and retinal ganglion cells (RGCs) are poorly understood. We have manipulated RGC density and availability of pre-synaptic tissie to examine their effects on the development of RGC dendritic arbors.

Visual field deprivation induces ocular enlargement, producing a larger than normal retinal area and a reduction in RGC density by 20-30%. Peripheral optic nerve section produces areas of RGC depletion with a mean reduction in density of 64%. In the former case, the ratio of pre-synaptic cells to RGCs remains constant, while in the latter the ratio is increased. This contrast allows us to separate the effects of RGC density from pre-synaptic area on RGC dendritic field size and area complexity.

In experimentally enlarged retinas, RGC dendritic arbors expand with ocular area in a compensatory manner that is consistent with simple stretching; arbor area was about 30% larger than normal, branch density was decreased, and arbor length was about 14% longer than normal. In RGC-depleted retina, dendritic fields of RGCs are larger than controls but do not fill the space available. Branch density of RGC dendrites in RGC-depleted areas is greater than in controls.

We conclude that both local RGC density and the availability of pre-synaptic terminal control RGC dendritic size and complexity. The limited growth of the RGC dendritic fields in areas with large space availability, together with the increase in arbor branches per unit area, suggest that pre-synaptic input is important in the shaping of RGC arbors postnatally.

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447.10


During divergence of retinal axons to both sides of the brain, both crossed and uncrossed filaments initially travel towards the midline for a period of several hours, and either cross the midline or turn back to ipsilateral optic tract, respectively (Godement et al., J. Neurosci., in press). To investigate how growth cones detect cues and which of several potential cues in the midline provide those cues, we analyzed the behavior of retinal growth cones from dorsal or ventral temporal retinas (source of crossed and uncrossed fibers) as they interact with chiasmatic tissue in vitro by video microscopy and DIC optics. When axons grow on a polylysine/laminin substrate, they advanced rapidly and had long lamellipodial extensions. Within 50 microns of a chiasm cell, growth cones slowed and became more filopodial. After filopodial contact, all growth cones paused in advance, with a single filopodium attached to the cell. Subsequently, the behavior of the two sets of growth cones differed. Uncrossed growth cones displayed a greater degree of collapse, did not grow onto the cells, and took longer to turn away from the cells. Retrospective immunocytochemistry will indicate the extent to which neural and non-neuronal chiasm cells inhibit uncrossed growth cones.

To further characterize the intracellular machinery in detection of cues, we are investigating filopodia-specific phosphoryryosine by using retrograde immunocytochemistry of identified growth cones and antibodies to phospho-tyrosine to elucidate the detection and sources of cues leading to retinal axon divergence.

447.12

DYNAMICS OF DENDRITIC DEVELOPMENT IN THE OPTIC TECTUM OF LIVE ZEBRAFISH EMBRYOS. R.J. Kaether* and C.A.O. Steurmer, Univ. of Connecticut, D-78445 Konstanz, Germany.

To visualize the development of dendrites in the zebrafish optic tectum, 0.5-30pL of the fluorescent dye DiO (in solution) were pressure-injected into the cell rich layer of tecta in 50-70hr old embryos. These cells reside deep to the tectal neurite into which retinal axons enter and where they form their terminal arbors (Steurmer, J Neurosci 8, 1988). Labelled cells and dendrites were viewed from above through the unopened skull of live embryos with low intensity light and a SIT camera (Hamaatsu), and monitored through a time lapse video recording system.

The tectal neurons were first visualized from both eyes to see different axon arbor subregional areas. Simultaneously they begin to develop their dendrites which grow in a radial orientation towards the developing neuropil. While elongating, the dendrites form branches with growth cone like structures and up to 15m long filopodia which radiate into all directions. As has previously been described for retinal axons (Kaether & Steurmer, J Neurosci 12, 1992), dendrites develop through the emission and retraction of filopodia and primary branches over periods to 14hr. With time some branches stabilize while others are entirely reabsorbed. Gradually, the dendritic arbors increase in diameter up to 30m in 90th old embryos. Thus, developing dendrites of tectal neurals exhibit an exploratory growth behavior similar to that of retinal axon arbors in the developing tectal neuropil. Retraction and extension of processes of dendrites coincides with the dynamic modeling of retinal axon arbors, suggesting that processes of both may contact each other and influence each other’s growth and shaping. Supported by DFG, SFB 156, TP6.

448.1

DEVELOPMENTAL EXPRESSION OF G PROTEINS IN THE EMBRYONIC NERVOUS SYSTEM OF MANDUCA SEXTA. P.E. Capponi, M. Michelle Rasmussen, and A.M. Horgan.

Ataxia L1,L2, Oregon Health Sciences University, Portland, OR 97201.

We are investigating the developmental role of G proteins in the formation of the CNS. This highly conserved family of intracellular signaling molecules is widely distributed in the nervous systems of all organisms. The developmental role of G proteins in developing nerves remains poorly defined. Using affinity-purified antisera against different G alpha subunits, we characterized the developmental expression of the major classes of G proteins (including proteins related to Go, Gi, Gt, and Gq) during the formation of the CNS. Whole mount immunohistochemical staining and protein immunoblots of embryonic tissue revealed that the different G protein classes appeared in precise, spatiotemporal patterns within the developing ganglia. Several G proteins were initially seen in subsets of undifferentiated precursors but underwent a subsequent phase of regulation as development progressed, suggesting that individual G proteins may serve a progression of functions during the formation of the nervous system. In particular, while several G proteins could be detected in the mature nervous systems of the CNS, only Go-dependent proteins could be detected in regions of axonal outgrowth. Preliminary experiments using aluminum fluoride to stimulate G protein activity caused a substantial inhibition of process outgrowth, suggesting that one function of G protein coupling is in pathfinding neuronal outgrowth. These results can now be used to examine more specific expression of individual or G proteins in the context of identified neurons within the embryonic nervous system. Supported by NSF BNS 9013059.

448.2


Multiple extracellular signals have been shown to contribute to neurite outgrowth and growth cone guidance. Such cues activate intracellular signal transduction pathways which in turn lead to changes in the size and shape of the growth cone. Additionally, second messenger systems are likely to influence the ability of the growth cone to interact with specific cell adhesion molecules. Protein kinases and phosphatases may play a crucial role as second messengers which regulate growth and migration. For these studies, mouse cerebellar cells were cultured on three different substrates; L-1, NCAM, and P84. Various protein and kinase phosphatases were then administered to the cells and the effect was monitored on growth cone morphology was assessed. Cells grown on L-1 or NCAM which were exposed to genistein, a tyrosine kinase inhibitor, had more filopodia, both at the growth cone and along the neurite. Treated cells also exhibited more side branching of the neurite. When the cells were grown on P84, the effect of genistein was less obvious and the cells resembled those in the control. Interestingly, growth cones on P84 normally have extensive filopodia. The lack of effect of genistein on cells grown on P84 may reflect a difference in the role played by tyrosine kinases in the growth of L-1 and P84 cells. Cells growing on L-1 were treated with okadac acid, a serine/threonine phosphatase inhibitor, the growth cones became more lamellipodial or sheetlike in appearance. Okadac acid-treated cells grown on NCAM seemed to have larger growth cones than the control cells. Okadac acid did not seem to have an effect on cells grown on P84. Each of the inhibitors affected the cells differently and the effect each drug was substrate dependent. These results support the idea that protein kinases and phosphatases are involved in the regulation of growth cone morphology. Supported by NIH grant EY05308.
448.3

DISTRIBUTION OF PROTEIN KINASE C (α, β, γ) SUBTYPES IN REGENERATING POSTERIOR ROOT SENSORY NEURONS. ORCUTT, Y.Hirasawa, A. Mizoguchi and C. Ide. Dept. Orthopedic Surg., Kyushu Pref. Univ. Med., Kyushu 802 and Dept. Anatomy, Kobe Univ. Sch. Med., Kobe 650. Protein kinase C (PKC) is an enzyme activated by diacylglycerol resulting from the receptor-mediated hydrolysis of inositol phospholipids and is involved in a variety of cellular processes, including signal transduction, regulation of ion channels and neurotransmitter release, control of cell growth and differentiation, changes in cell morphology, and gene expression. In the present study, immunolocalization of PKC (α, β, γ subtypes) was studied immunocytochemically in the normal nerve fibers and in the regenerating sprouts (growth cones) from roots of Pannier following crush injury in rat peripheral nerve. Using Sprague-Dawley male rats weighing 200-250 gms were ligated for 24 h and unligated 24 h prior to sacrifice. The sciatic nerve was removed, postfixed with paraformaldehyde in PBS. The injured nerve segment was removed, embedded in OCT compound, quick-frozen, and cut on a cryostat. The sections were immunostained with anti-PKC α, β, γ subtypes with peroxidase and DAB. For electron microscopy, some selected sections were dehydrated in a graded series of ethanol, followed by OY-1, and embedded in Epon 812. Ultrathin sections were made and observed on an electron microscope. In the normal nerve, PKC (α, β, γ) immunoreactivity (IR) was present in all myelinated and unmyelinated axons. Electron microscopy showed that IR was localized on the acelin and in a part of axoplasm. In the injured nerves, growth cones extending through the crushed area exhibited intense and diffuse PKC (α, β, γ) IR in the axoplasm. Thus, PKCIR in growth cones demonstrates a pattern of distribution that differs from that of the parent axon, suggesting that PKC may have important functional roles in axonal sprouting and in the regulation of growth cone activity in the peripheral nervous system.

448.5

LOCALIZATION OF RYANObine RECEPTORS AT GROWTH CONES OF CULTURED NEURONS*1*, l. Bae,*, L.L. Suki2 and M.H. Ellisman1 1San Diego Microscopy & Imaging Resource, Dept. Neurosciences, Univ.Calif.San Diego, La Jolla; 2Dept. Pharmacology, Univ. Nevada, Reno. In a previous investigation in chick cerebellum, a developmental change in the distribution pattern of the beta or cardiac form of the ryanodine receptor (RyR) was observed (Oliver et al., 1993, Soc.NAT. Acad. SCI., 1993:90:2263). Immunolabelling for the beta or cardiac form of the RyR showed a decrease in a staining pattern associated with parallel fibers in the inner nuclear layer of the cerebellum in the second week posthatch. The change might be related to neurite extension in the molecular layer, where the increase may be required to regulate the intracellular calcium storage sites for the growth process. Also, since ryanodine receptor from caffeine-sensitive intracellular storage sites was reported to be crucial in Nl-35-evoked growth cone collapse (Bandow et al., 1993, Science 259:80-3) and the RyR is a calcium release channel sensitive to caffeine, we decided to examine the RyR distribution within growth cones. Using a monoclonal antibody 34C, which recognizes all RYR isoforms in chicken, RyR was immunoreactive within and growth cones of growth cones of chicken ciliary ganglion neurons and dorsal root ganglion neurons in primary cultures. Labelling was mainly concentrated in the lamellipodium of the growth cones, but not in the fine filopodia. The pattern of labeling was similar to that observed with alpha-tubulin in double-labeling experiments. This result suggests that the RyR gated calcium storage sites may be associated with the microtubule based cytoskeleton and/or transported to growth cones along microtubules. The IRs and calcium: SR Ca2+; RYR, the two proteins involved in intracellular calcium regulation, also exhibited a similar distribution to the RYR. The presence of RyR and other calcium regulatory proteins in growth cones suggests that calcium released from intracellular storage sites may play an important role in the regulation of growth cone dynamics.

448.6

INTERCELLULAR ASSOCIATIONS STABILIZE MORPHOLOGICAL PLASTICITY AND CULTURED RABBIT RHYNCHOCEREBRUM IN HAMSTER CONE NEURONS. C.V. Williams*, P.J. Mezey, S.B. Katz. Dept. Anatomy & Neurobiology, Colorado State University, Fort Collins, CO 80523. We have previously found that local changes in calcium can result in local morphological changes (i.e. filopodia induction) in isolated Helixema neurons. The present study asked if: 1) local calcium rises can modify dendritic morphology of isolated hippocampal pyramidal neurons in culture and 2) intercellular associations alter the magnitude of calcium rise and modify dendritic plasticity. Using a local electric field, both local rises in intracellular calcium and filopodial extensions were induced from dendritic shafts of isolated hippocampal neurons. Since previous studies suggest that contact with glial cells alters calcium homeostasis, we asked if such intercellular associations would indeed alter calcium responsiveness and morphological plasticity in dendritic shafts. The average per-unit length of the dendritic calcium levels obtained in dentrites was nearly 2-fold lower in neurons contacting other cells than in isolated neurons. Irrespective of the type of contact (i.e., via axons or dendrites), neurons contacting other cells show a smaller degree of filopodium induction in comparison with isolated neurons. These results indicate that factors which regulate calcium responsiveness may also regulate morphological plasticity. Local rises in calcium, such as induced in the present study could occur in vivo via presynaptic inputs or via the release of neurotransmitter from approaching axons during development. Subsequent morphological changes could lead to altered neuronal connectivity; in several cases, induced dendritic filopodia became new arbors in the dendritic tree. Thus tight regulation of intracellular calcium may be crucial for the establishment, maintenance and modification of neuronal architecture and the resultant neuronal connectivity.

448.7

INHIBITION OF PI 3-KINASE BLOCKS NERVE GROWTH FACTOR STIMULATED NEURITE OUTGROWTH AND MAINTENANCE IN PC12 CELLS. J. Blader, T. Jackson, C. Vergara, A. Wolf, L. Fraser, L. Hammond-Odie, and A. Theibert, Neurobiology Research Center/Department of Cell Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294 and "Babraham Institute, Cambridge UK CB2 4AT. In response to NGF, PC12 cells undergo morphological and biochemical changes, including neurite outgrowth and, differentiating into sympathetic neuron-like cells. NGF activates tyrosine kinases, phospholipase C and arachidonic acid release, leading to activation of PI 3-K, ras and MAP kinase pathways. NGF also stimulates phosphoinositide 3-kinase (PI3K), which phosphorylates PIP2, resulting in rapid, sustained increases in phosphatidylinositol 3,4,5-trisphosphate (PIP3). We have used an inhibitor (LY294002) of PI3K to investigate the role of PI3K in NGF signalling. Wortmannin (WM) inhibited NGF-stimulated increases in PIP3 by direct inhibition of the catalytic activity, two other proteins involved in PI3K regulation, also inhibited a similar distribution to the RYR. The presence of RyR and other calcium regulatory proteins in growth cones suggests that calcium released from intracellular storage sites may play an important role in the regulation of growth cone dynamics.
SYNAPTIC pregnagliconic growth cones collapse and retracted when they con- tact the processes of dorsal root ganglion neurons (DRG) in vitro (Moorman and Hume, 1990). The signals that trigger this behavior and the signal transduction mechanisms are unknown. In the present study we used time lapse video microscopy to determine whether inhibitors of specific second messenger systems prevent the collapse of pregnagliconic growth cones when they directly contact DRG neurites.

Pregangliocnic neurites were retrogradely labeled by DiI injections into the sympathetic chain of stage 30 chick embryos. Sixteen to 20 hours after the medial graft containing the labeled pregangliocnic neurites was cut into small pieces and placed as explants on a laminin-coated substrate. After allowing several hours for these cells to extend neurites, DiO labeled DRG's were added to the dish and time lapse images were acquired. We examined pregangliocnic growth cone-DRG neurite interactions in the presence of pertussis toxin (100 ng/ml) or omega conotoxin GVIA (5 uM). Cultures were pre-incubated in pertussis toxin for at least 2 hours prior to observation.

Each culture studied, both surface area and length measurements were made at regular time intervals.

Our results show that pertussis toxin, a blocker of G protein activation, and omega conotoxin, a blocker of N-type calcium channels, inhibit the collapse of pregangliocnic growth cones when contacting DRG neurites. These findings suggest that calcium and G protein may both play a role in mediating growth cone collapse in pregangliocnic growth cones.
449.1


W. Halfter1 Dept. of Neurobiology; University of Pittsburgh

In the early embryonic retina optic axons grow toward the optic disc and form a centripetal axon pattern. To address the question whether the directed growth of axons results from an attraction by the optic disc or from repulsion by the retinal periphery pieces of donor retina were grafted to ectopic sites of 3 organ-cultured eyes. After 24-48 hrs the eyes were studied for alteration in the axonal pattern as a result from the transplants. 1. Peripheral donor retina grafted into the central position of the host are non-permissive for growing axons. 2. The grafting of an optic disc to an ectopic site of the host and the removal of the original optic disc caused a major repulsion of axons to the grafted optic disc. 2. Axons encountering the grafted optic disc left the optic fiber layer to exit the eye. The experiments demonstrate that the centripetal pattern of axons in the retina is caused by a repulsion of axons by the retinal periphery and by a local attraction by the optic disc. The optic disc operates as an exit for the axons from the eye into the optic nerve.

449.2

CHONDROITIN SULFATE INTERFERES WITH RETINAL GANGLION CELL PATHFINDING IN THE VISUAL SYSTEM OF THE ECORDATE, XENOPUS LATEXICALIS A. Walé and C.E. Holt Dept. of Biology, UCSD, La Jolla, CA 92030-0357

The axons of developing retinal ganglion cells (RGC) navigate along a defined route to reach their target, the optic tectum. Pathfinding is mediated by specific interactions between the growth cones and the substrate presented by the local environment. We investigated the putative involvement of chondroitin sulphate (CS) in the development of the retinotectal projection.

Exposed brain preparations were used to investigate the influence of free CS on the developing optic projection during the period when RGC axons grow from the optic tract to the tectum (stages 31/34 to 40) and axons were visualized with anterograde HRP at stage 40. In contrast to other well applied glycosaminoglycans, CS (200μg) showed a dramatic effect on pathfinding. The tract was highly broadened with axons growing virtually over every area of the telen- diencephalon. These widened tracts were not shorter and growth cone morphology did not appear to be different compared to controls. Immunolabeling revealed that normal CS expression in the neuropil overlapped with RGC axon staining showing that RGC axons grow on a CS rich substrate. Exposed intact eyes grown in culture on a laminin (LN) substrate with CS (1mg/ml) added to the medium did not show any change in either neurite outgrowth or elongation. However, when explanted eyes were grown on a LN/CS substrate, a dose dependent inhibitory effect on neurite outgrowth was observed, suggesting that even though CS does not inhibit growth per se, it may direct growth toward lower CS concentrations in the embryo during pathfinding. Chondroitinase ABC and collagenase were used to create CS free embryos and the pathfinding behavior of RGC axons was examined.

(Supported by NIH #NS23780 and PEW Scholars Award)

449.3

bFGF DISRUPTS TECTAL RECOGNITION IN THE DEVELOPING VISUAL SYSTEM OF XENOPUS LAEVIUSCULUS McFarland1 and C.E. Holt. Dept. Biology, Univ. of California San Diego, La Jolla, CA 92093.

We are investigating the molecular cues that develop retinal ganglion cell (RGC) axons of Xenopus laevis use to recognize and migrate to their target. Since growth factors are known to affect neuronal differentiation, are spatially and temporally regulated in the developing brain and are expressed in the target cells of some neurons, it is possible that they may be involved in these recognition events. The fibroblast growth factor (FGF) family and their receptors have been well studied in the visual system; therefore, we have investigated the role of one member, basic FGF (bFGF), in target recognition.

A recombinant form of Xenopus bFGF (XbFGF) was applied to exposed embryonic brain preparations during the period when RGC axons grow from the optic chiasm to the tectum (stage 33/34 to 40). Axons were visualized with HRP at stage 40. XbFGF (10nM) severely affected retinotectal targeting. In the majority of cases (94%, n=19), axons failed to enter the tectum, instead, they skirted around the diencepithelial/ectodermal border; growing dorsally towards and occasionally over the dorsal midline, and ventrally toward the tectum. We performed timing experiments in an attempt to elucidate XbFGF's action. First, XbFGF was applied at stage 33/34 for 2 hours. This early phase was sufficient to disrupt target recognition (90%, n=19). Consequently, if XbFGF application was delayed until stage 37/38, when the first RGC axons usually reach the tectum, target recognition was essentially normal. These results indicate that XbFGF needs to be present early on, for a short period of time, to exert its effects. Immunostaining shows that ubiquitous XbFGF binds to nerve fibers in the developing brain, including RGCs. bFGF acts on the RGCs themselves. These findings suggest that bFGF may play a role in target recognition.

(Supported by NIH #NS23780, PEW Scholars Award and MRC of Canada)

449.4

PERTURBATION OF G-PROTEIN FUNCTION AND RETINAL GROWTH CONE BEHAVIOR. C.S. Chien1, A.T. Benedikt and W.A. Harris. Dept. of Biology, UC San Diego, La Jolla, CA 92030-0367

Heteroteric G-proteins are ubiquitous intermediates in signal transduction which are enriched in growth cone membranes. We are studying their possible function in the guidance of Xenopus retinal growth cones both in vitro and in vitro.

In culture, the wesp venom peptide mastoparan (MP), which directly activates G proteins, has been shown to cause growth cone collapse (Igarashi et al., 1995 Sciencem 259:77). Furthermore, this collapse was prevented by pretreatment with pertussis toxin (PTX), which inactivates G proteins by covalent modification. We have obtained similar effects with Xenopus retinal growth cones in culture, 10μM MP applied for 30 min XMP (5.5mM) severely affected retinotectal targeting. In the majority of cases (75%, n=20), axons failed to enter the tectum, instead, they skirted around the diencepithelial/ectodermal border; growing dorsally towards and occasionally over the dorsal midline, and ventrally toward the tectum. We performed timing experiments in an attempt to elucidate XbFGF's action. First, XbFGF was applied at stage 33/34 for 2 hours. This early phase was sufficient to disrupt target recognition (90%, n=19). Consequently, if XbFGF application was delayed until stage 37/38, when the first RGC axons usually reach the tectum, target recognition was essentially normal. These results indicate that XbFGF needs to be present early on, for a short period of time, to exert its effects. Immunostaining shows that ubiquitous XbFGF binds to nerve fibers in the developing brain, including RGCs. bFGF acts on the RGCs themselves. These findings suggest that bFGF may play a role in target recognition.

(Supported by NIH #NS23780, PEW Scholars Award and MRC of Canada)
449.5

CHICK RETINAL AXONS BRANCH PREFERENTIALLY ON MEMBRANE MEMBRANE-DERIVED AXONAL GUIDANCE TOPOGRAPHICAL APPORTCULAR TUM. A.J. Roskite* and D.D.M. O'Leary.

Molecular Neurobiology, Lew Faculty, La Jolla CA 92037.

Many single, position-independent axons are hypothesized to guide or restrict growing retinal axons to topographically appropriate sites in their central targets. In vivo studies in chick and rat indicate that retinal axons form topographically ordered arbors by the extension of collateral branches along their length. We have recently reported that E18 rat retinal axons grown perpendicular to membrane fragments derived from rostral and caudal E18 superior colliculus (SC) branch preferentially on rostral SC membranes due to an inhibitory molecule PI-anchored to SC membranes.

To determine whether chick retinal axons exhibit a branching specificity similar to that found for rat, we have grown E6 chick retinal explants on compartments of alternating lanes of membrane fragments from rostral and caudal E9 chick retina. Temporal chick retinal axons show a consistent branching preference for their topographically appropriate retinal membrane planes. Nasal axons show no consistent preference. Time-lapse videomicroscopy of the stripe assay cultures reveals that branches form interstitially along the axon shaft, as they seem to in vivo, as well as grow cone bifurcation. Thus, in chick, as in rat, the topographic bias in retinal axon branching observed in vivo may be controlled by membrane bound molecules. Further time-lapse microscopy of these cultures will allow us to determine whether the observed branching preference is a result of substrate-influenced branch initiation or branch stabilization.

449.7

TOPOLOGICAL SPECIFICITY IN REINNERRATION OF THE SUPERIOR COLICULUS BY REGENERATED RETINAL GANGLION CELL AXONS IN ADULT HAMSTERS. ROBERT D. Sauvé, Haime S. Vincent.

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In rodents, regenerating retinal ganglion cells (RGCs) project their axons to caudal and rostral contralateral superior colliculus (SC) respectively. We have examined the projections of RGC axons regenerated through unbranched pedicles in the adult hamster retina. Projections were recorded from the superior colliculus (SC) and the contralateral SC in adult hamsters. Responses to visual stimulation were recorded from terminal arbors of individual regenerated RGC axons and the postsynaptic neurons with which they make synapses in the retina (SC) and the contralateral SC. RGC positions in the retina and the corresponding position to these terminal arbors were inferred from the location of their visual receptive fields on a tanger screen. In 6 to 13 RGCs with projections to the SC were identified in 11 animals 30 to 60 days after injury.

At a given site in the SC, recordings could be made from axon terminal arbors emanating from widely separated RGCs. Conversely, nearby RGCs could project to widely separated sites in the SC. The relative position of each possible pair of RGCs was determined with respect to the position of the terminal arbors of the axons of previous site visits. These RGCs were inferred from the caudal dorsal displacements of co-cultured site responses in the SC. Paired were excluded as ambiguously oriented if the direction of displacement fell beyond ±45° from the caudal dorsal axis in the SC. Of the 101 available pairs of RGC projections, 622 had appropriate and 394 inappropriate caudal dorsal displacements in the SC. Although normal retinotectal topography is not reproduced, this 3:2 preference for appropriate projection suggests that RGC axons may recognize caudal dorsal position cues as they reinnervate the SC.

449.8

AN ANTIGEN IN THE OPTIC PATHWAY IS EXPRESSED IN EYELESS ANIMALS. Nielsen Fernandez and Sally Hoskins.

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To analyze the molecular axis guidance in the developing optic nerve of the frog Xenopus laevis, we made monoclonal antibodies to optic tract tract-less fragment and produced gk1, which blocks the optic nerve projection to the optic tectum beginning at embryonic stages. As reported previously, the pathway to the thalamus and the thalamic target zones are not retained by gk1, but the possibility that the gk1 antigen plays a role in optic axon target selection.

To examine whether all of the staining seen in the optic pathway was associated with the optic nerve, we deleted optic cups from early embryos, and assessed gk1 immunoreactivity in cryosections of eyeless tadpoles at tadpole stages. Eyeless tadpoles showed gk1 immunoreactivity in the position normally occupied by the optic chiasm, as well as in a line of cells marking the position of the optic tract along the wall of the diencephalon, and in the optic tectum. We conclude that the gk1 antigen is associated both with the developing optic nerve and with its pathway, raising the possibility that early gk1 expression in the optic pathway marks the route taken by the subset of retinal ganglion cell axons that projects to the optic tectum.

In experiments in progress will establish how early the pathway-associated gk1 appears.

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449.9

THE ORIGIN AND COURSE OF RETINOFOGAL AXONS DURING NORMAL DEVELOPMENT OF THE FERRET, G.E. Baker and R.C. Gilchrist (SPON: Brain Research Association, Department of Human Anatomy, University of Oxford, South Parks Road, Oxford OX1 3QX, UK.

Developing retinal ganglion cell axons grow into either the contralateral or ipsilateral optic tract. We have studied the course of these axons in ferrets to examine their development and determine if their prechiasmatic organization at any age might predispose them to a crossed or uncrossed course at the optic chiasm.

Pregnant ferrets were deeply anaesthetized and fixed with 4% paraformaldehyde. Retinal implants of the tracer Dil were made to label axons from one eye, and unilateral optic tract tracts were made to label the uncrossed axons and ganglion cells of the other eye. The distribution of axons was observed in mice and rats (Goodman et al., Development 101, 515; Chan et al., JNC 363, 362). The ipsilaterally-projecting cells have no apparent preferred location within that region. Behind the eye, the uncrossed axons became increasingly concentrated laterally closer to the optic chiasm where few are found in medial locations. A narrow ventromedial (VT) core of ipsilaterally-projecting cells is first observed at E28 and broadens as axons regrow in the dorso-medial retina. The distribution of axons is observed at this and later age contrasts with the earlier stages and resembles that of the adult; postocularly the majority of uncrossed axons are concentrated ventrally, becoming more dispersed along the course of the nerve. As the chiasm many are found in a medial location. At no age are the uncrossed and crossed axons segregated prechiasmatically.

We conclude that spatial location in the prechiasmatic nerve may determine the uncrossed path of the earliest fibres but is not relevant for the later VT component.

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449.11
SUBTRACTIVE cDNA LIBRARY SCREEN FOR GENES UPREGULATED OR DOWNREGULATED AFTER DEAFFERENTATION OF THE RAT SUPERIOR COLICULUS. A.D. Crawford\textsuperscript{1}, F. Bonhoeffer\textsuperscript{1}, and M. Bibb\textsuperscript{2}. Max-Planck-Institut für Entwicklungsbiologie\textsuperscript{1} and Neurobiologische Universitätsklinik\textsuperscript{2}, Tübingen, Germany.

During development of the vertebrate visual system an orderly projection of retinal ganglion cells onto the superior colliculus is established. The molecular mechanisms that might govern this process are still unknown, but probably include the coordinated interaction of target-specific axon guidance molecules and their corresponding growth cone receptors. Until recently, it was believed that in higher vertebrates, these molecules are only expressed during the actual formation of the retinocollicular projection. However, it was recently shown that cell-surface guidance activities that are not normally detectable in the superior colliculus (SC) of the adult rat temporarily display deafferentation of the SC through extorision of the optic nerve (Wenzelmann et al., 1993). We have initiated a subtractive cDNA library screen to clone the genes encoding these guidance molecules, or possibly these molecules that are responsible for making the cells in the normal adult. The subtraction protocol involved multiple rounds of hybridization of cDNA from deafferented SC with an excess of biotinylated cDNA from normal SC, and vice versa, each round followed by extraction with streptavidin and PCR amplification of the remaining cDNA (Wang and Brown, 1991). The resulting subtracted cDNA libraries are one highly enriched for genes that are upregulated after deafferentation, the other for downregulated genes, were then differentially screened to identify these upregulated and downregulated clones. In an initial pilot screen of about 250 clones from each library, 16 upregulated and 7 downregulated clones were identified. These clones are currently being subjected to sequence, Northern, and in situ analysis. In addition, full-scale screens of about 5000 clones from each library are currently in progress. The differences in expression in normal versus deafferented SC of these clones isolated to date varied from 2-fold to at least 10-fold, with 2 upregulated clones showing no detectable expression in normal SC.

449.13
PRECOCIOUS INVASION OF THE OPTIC STALK BY TRANSIENT CENTRIFUGAL AXONS IN THE FERRET. B.R. Reese\textsuperscript{1} and S.F. Celler Neuroscience Research Institute and Departments of Psychology and Biology, University of California at Santa Barbara, CA 93106-5060.

The present study demonstrates 1) that the fetal optic nerve contains a conspicuous population of centrifugal axons; 2) that the cells of origin are situated in both the ventrolateral diencephalon and in the hypothalamic region; 3) that the projection is transient, and never invades the retina itself; and 4) that these centrifugal axons evade the optic stalk prior to the arrival of the retinal axons. Fetal ferrets (E-22 through E-30) were fixed with 4% paraformaldehyde and examined using DiI to trace the retinotectal and centrifugal pathways. In addition, we used E-tubulin immunocytochemistry to detect the differentiation of the first axons within the retina and optic stalk. Dye implants into the optic nerve head, but not the retinal periphery, retrogradely label somata in the ventrolateral diencephalon, provided the implants are made in fetal brains. Likewise, dye implants into this region of the diencephalon, rostral to the optic tract, anterogradely label centrifugal axons that course through the normal optic pathway, but never enter the retina. The centrifugal axons course retrogradely from their cells of origin as 2-5 fascicles and turn laterally to enter the optic nerve where they emerge from the hypothalamus. Some of the fascicles course extra-cerebrally for a short segment at this junction, as described by Guillemé and Weidner (J. Comp. Neurol. 1987a, 265, 203; 1987b, 265, 218).

The optic nerve head and retinal fiber layer are immunoreactive for E-tubulin on E-24 and thereafter, whereas on E-22, they are immunonegative. Yet immunopositive fascicles of axons course from the ventral diencephalon into the optic stalk on E-22, confirming the precocious nature of the centrifugal projection. These fascicles are identical in trajectory to the centrifugal axons which are still readily labelled by dye implants into the future optic nerve head on E-22. These same implants on E-25, and only on E-22, also label cells in the future chromatogenic region, situated immediately ipsilateral to the midline.

These centrifugal axons may serve a transient, guidance function for later developing optic axons. The cells situated at the midline may additionally play a signalling function for optic axons as they evade the optic plate. The existence of these centrifugal axons complicates the identification of early-developing axons in otherwise unlabeled tissue.

FORMATION AND SPECIFICITY OF SYNAPSES III

450.1
FASCIKLIN III AS A SYNAPTIC TARGET RECOGNITION MOLECULE IN DROSOPHILA. A. Cuda\textsuperscript{1}, P. Stase\textsuperscript{1} and J. Hsiao\textsuperscript{1}. Dept. Physics, Univ. Tokyo, Tokyo, Japan, & Dept. Biol., SUNY, Albany, NY.

Motor neurons RPS consistently innervates muscles 6 and 7 in Drosophila embryos. During synapticogenesis, the cell surface glycoprotein fasciclin III appears in both the RPS and muscles 6 and 7. As fasciclin III has shown to be a homophilic adhesion molecule, such expression raises the possibility that it may function as a specific "target recognition molecule" for RPS. We have tested whether fasciclin III suffices to select its target choice in vivo by examining the effects of deletion or missexpression of this molecule as assessed by intracellular dye injection and immunocytochemistry. First we have found that in the existing fasciclin III null mutant RPS reliably forms synapses with its normal targets. Next, we have generated transgenic flies which misexpresses fasciclin III ectopically on all skeletal muscles during neuramorphogenetic synthesis. This was accomplished by inserting a heat-inducible fasciclin III gene under the control of the Myosin heavy chain promoter, and transforming this construct into flies by P-element mediated transgenesis. In these transformant flies, we have observed that RPS does innervate non-target muscle cells while other myoneurons (e.g. RPI) innervate their targets normally. Therefore, this provides single cell-level evidence supporting that fasciclin III functions as a specific "target recognition molecule" for a specific set of target neurones. Furthermore, taken together with the analysis on the null mutation, the results also suggest that fasciclin III is one member of a set of functionally redundant recognition molecules which are involved in an "either/or" manner during target recognition by RPS. Controlled misexpression of such recognition molecules is an effective approach to determining the functional roles of these proteins in vivo, which may be difficult to confirm by analysis of null mutations alone.

450.2
MOTONEURONS DISCRIMINATE APPROPRIATE FROM NOVEL TARGETS DURING SYNAPSE FORMATION IN CULTURE. J.C. Peyer and M.J. Zirner\textsuperscript{1}. Department of Biology, Texas A&M University, College Station, TX 77843.

Motor neuron B19 innervates the supraspinal radial somus (SLrT) muscle of the Helisoma bacular musculature. This neuromuscular synapse reforms in culture and its regeneration requires target-dependent induction of secretion capabilities. The release of the normal neurotrophin underlying this induction involves modification in the presynaptic secretory function such that action potentials, uncoupled from secretion prior to contact, evoke neurotrophic release following SLrT-contact muscle contact. Previous studies have shown that novel target muscles, not typically tested by B19, vary in their ability to induce secretory changes. For example, contact with superficial radial tendon (SMrT) muscle causes an enhancement of secretory capabilities while contact with foot (POD) muscle fibers does not. Based on these results, we hypothesize that B19 has the capacity to recognize specific muscle fibers and that muscle contacts trigger different presynaptic secretory responses. Our hypothesis maintains that mechanisms responsible for these differential changes are likely to be related to temporal aspects of axon sprouting. To further test the ability of B19 to discriminate between muscle targets, dual target contact experiments were performed where single B19s were cultured in contact by two muscle partners. After 3 days of contact, rates of spontaneous synaptic potentials (SSPs) and the presence of evoked synaptic potentials were assayed. In B19-SLR/SMT preparations (n=24), SSP rates derived from SLrT and SMrT-contacted neurites were 9.6 and 1.6 SSPs/min, respectively. Evoked release was detected from 20% of SLrT and 19% of SMrT neurites. In B19-SLT/POD preparations (n=15), SSP rates derived from SLrT- and POD-contacted neurites were 3.0 and 1.1 SSPs/min, respectively. Evoked release was detected from 50% of SLrT- contacted neurites and none of POD-contacted neurites. Evoked release was not observed in SMT- or POD-contacted neurites, suggesting that specific neuron-muscle contacts can regulate secretory properties of motorneuron B19 in a cell-specific manner.
ACTIVITY REGULATES THE SEGREGATION OF PRESYNAPTIC INPUTS ON A COMMON POSTSYNAPTIC TARGET IN VITRO. Z. Sun* and S. Schacher. Ctr. Neurobiol. & Behav., Colby Univ. College of P & S, NYSPI, New York, NY 10032. Previous studies indicate that Aplysia sensory neurons may 'compute' with one another in establishing chemical connections with a single motor target in vitro. Although the sensory cell displays no spontaneous synaptic activity, they generate their varicosities to different portions of the motor axons over time. The functional consequence is a cell number-dependent decrease in the average SRSF evoked by each sensory cell. We examined whether adding activity known to evoke a facilitatory response in mature connections—increased in CaM levels via Xenopus steric or tetric stimulation (10 Hz for 4-5 sec)—during the early stages of synapse formation would modulate the intrinsic segregatory process. Two sensory cells were cocultured with one motor cell and examined on day 2 and day 4 for changes in synaptic efficacy and structure. Two significant differences are the functional and structural changes for each sensory cell over time with control axons or following stimulation of the motor cell. Injection of cAMP or a static stimulation of one sensory cell significantly increased the amplitude of the evoked SRSF and varicosities of the treated cells compared to their respective control sensory cells in the same culture. Increasing cAMP levels appeared to increase the rate of new varicosity formation by the injected cell while tetanus also evoked an increase in the number of existing varicosities of the control unstimulated cell. The synaptic effect may be mediated by co-cultivation depolarization in the motor cell. Paracrine stimulation in both the motor and one sensory cell resulted in a larger difference in the functional and structural development by the two sensory cells. These results suggest that different forms of activity may regulate specific aspects of the segregatory process—formation or elimination of synapses—associated with the fine tuning of connections formed by converging presynaptic inputs on a common target.

DIFFERENTIAL EXPRESSION OF G-PROTEINS IN IDENTIFIED LIMNOEA NEURONS MAY DETERMINE TARGET CELL SELECTION AND SPECIFICITY OF SYNAPTIC CONNECTIONS. Gianna Spada, N.J. Spada, and Lokwisi, K. Departments of Anatomy and Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1. We have recently developed a new in vitro model system to study mechanisms underlying target cell selection and specificity of synaptic connections in the gastropod Lymnaea stagnalis. Of the identified dopaminergic interneuron termed Right Pedal Dorsal 1 RPD1 multiple monosynaptic connections with a number of target cells (Right Pedal A) in vivo and these connections are re-established in vitro. RPD1 does not synapse with non target cells (Left Pedal A) either in vivo or in vitro, despite the presence of functional dopamine receptor on these cells. In the present study, we therefore hypothesized that differential G-protein couplings to the dopamine receptor may determine target selection by RPD1 in vitro. We first determined whether the effects of exogenous dopamine on both target and non target cells were mediated through different G-proteins. We used the bacterial toxins, Pertussis toxin (PTX), which is known to inhibit Gi and Go. Both target and non target cells were incubated for 18-24 hours with 1 nM PTX (n=7), whereas the similar inhibitory responses of the target cells were abolished (n>5). These data lend support to the hypothesis that G-proteins may play an important role in determining the specificity of target cell selection by Lymnaea neurons.

TIMING OF AFFERENT INGROWTH AND TARGET-DERIVED STOP SIGNALS IN DEVELOPING CEREBELLAR G. Zhang, R. Blazekaci, and C. Mason. Dept. Pathology, Coll. Phys. Surg., Columbia, University, NY 10032. Our previous work has demonstrated a "stop-growing signal" derived from target cells, based on the behavior of pontine mossy fibers and granule cells from mouse cerebellum in vitro (Baird et al., 1992, J. Neurosci. 12:619). To further investigate the nature of the stop signal and its specificity (Baird et al., 1992, J. Neurobiol. 23:579), we examined afferent-target interactions in two situations: when afferents arrive before target cell availability, and when the available target cells are more mature than usual. First; vestibular mossy fiber ingrowth, position and contacts were studied by DiI labeling, in slices prepared in conjunction with calbindin anisera to label Purkinje cells, and electron microscopy of photoconverted DiI-labeled terminals. At E17, vestibular fibers extend well into the emerging cortical layers in lobule X. Vestibular fibers with small growing tips terminate amongst the Purkinje cell zone and on the underside of the external granule cell layer. Immature contacts are made primarily on Purkinje cell somata, not on dendrites, and switch to granule cells after the first few postnatal days. Second, explants of pontine nuclei were cocultured with purified granule cells, which were aged in vitro, or taken from different aged animals. After P11 (or the equivalent days in vitro), less than half of the explants exhibited short neurites, compared to about 90% at earlier ages, indicating lack of or waning of a stop signal. These results point to temporal and spatial orchestration of signaling events on target cells. (Supported by NS 15961 to C.M.)

SYNAPSIN-LIKE IMMUNOREACTIVITY IN INVERTEBRATE NEURONS IN VITRO: REDISTRIBUTION FOLLOWING THE ESTABLISHMENT OF SYNAPTIC CONTACTS. G. Cerilli, F. Benfanti*, M. Ghiradelli*, F. Vitello, and P.G. Montecucco. Inst. of Human Physiology, University of Bari, Dept. of Experimental Medicine, University of Rome "For Vergate", Dept. of Human Anatomy and Physiology, University of Turin, Italy. The characterization of proteins involved in the storage and release of neurotransmitters has been a major step in understanding the synaptic function. Among them, the synapsins, a family of evolutionarily conserved, neuron-specific phosphoproteins associated with the specialized small synaptic vesicles, appear to play a pivotal role in both mature and developing nerve terminals. Neuronal cultures from invertebrate nervous system represent a model particularly suitable to analyze the synaptic functions; in fact, in these cultures presynaptic and target neurons form active contacts which can undergo synaptic plasticity. We studied the redistribution of the synapsin-like immunoreactivity in identified Dusophy and Helix cultured neurons following the establishment of synaptic contacts. To aim we cocultured a large serotonergic cell (GCG) from the cerebral ganglion and its target neuron (B2) from the buccal ganglion; on the other hand, the use of cell markers can be electrophysiologically tested. After different periods in culture we localized the synapsin-like immunoreactivity as a result of the target cells and measured the improvement in the presynaptic cell substantially overlapped the serotonin immunostaining (used as a marker of active synapses). On the contrary, in the absence of the physiological target a diffuse labelling pattern along the outgrowing neurites was observed. This is in good agreement with the data showing that in cultures of hippocampal neurons of mammal synaptic vesicles cluster in presynaptic terminals following contacts with dendrites of other neurons. Our results suggest that invertebrate cultured neurons the sorting of synaptic vesicle proteins to the presynaptic ending is strongly affected by the presence of the target cell.
TARGETING OF FUNCTIONAL SUBSETS OF NEURONS MEDIATED VIA THEIR AXONAL CARBOHYDRATE MARKERS. B. Zisser and J. Song, Dept. Physiol., Michigan State University, East Lansing, MI 48824

Sensory information is commonly channeled into multiple target regions in the CNS. Some of these target regions are exclusively innervated by just one sensory modality while other target regions are multimodal, receiving input from several sensory modalities. There is increasing evidence throughout phylogeny that neurons conveying different sensory modalities are chemically ecoded with some glycoconjugates. We investigated to what extent two such carbohydrate markers mediate the targeting of their respective axonal subsets in the CNS of the locust. Using [3H]glucosamine and [3H] mannose, we found that the targeting of each axonal subset in the synaptic neuropil was perturbed only by Fab fragments binding to the subset's own carbohydrate markers. These observations elucidate the nature of the interactions mediated by these two carbohydrate markers with glycosidases and neoglycoproteins indicated that the targeting of putative mechanosensory cells is mediated by a galactose-specific recognition; in contrast, the targeting of putative chemosensory markers is mediated mostly by a glucose-specific recognition with some galactose component. The different interctions of the two neuronal subsets, one mediated exclusively by galactose and the other one mostly by glucose with a minor galactose component, are consistent with the observation that their projections show partial overlap in the synaptic neuropil. Thus, the intact-axon terminal model system to study the normal function of neuronal carbohydrate markers in synaptic connectivity.

MORPHOLOGICAL AND NEUROCHEMICAL TARGET PREFERENCES OF ADULT PHOTORECEPTORS REGENERATING IN VITRO. D.M. Sherr* and E. Townes-Anderson, Cornell U, Ithaca, NY, 14850

The specificity of regenerative synaptogenesis by adult vertebrate neurons is poorly understood. Contact formation between pharate neurons and second- and third-order retinal neurons with identified amino acid content was examined by culturing differentiated neurons isolated from salamander and goldfish embryos. In the salamander system, neurons retain characteristic morphological features and regenerate synapses. Regenerating photo-receptors form presynaptic varicosities that contain synaptic vesicles and the transmitter glutamate; 134 varicosities have been analyzed. Varicosities usually contacted body cells (82.3%) rather than processes. Photoreceptors had the opportunity to interact with all three class of retinal neurons, but contact was uniformly to improper targets: multipolar cells (32.8%), includes amacrine, ganglion and interplexiform (5.7%), than proper targets: bipolar (0.7%), horizontal (0.7%) or photoreceptor cells (5.2%). However, most contacts (60.4%) were onto cells that had lost processes during isolation and could not be identified unequivocally, but their morphology and growth were inconsistent with photoreceptor or horizontal cells. Single-label immunocytochemistry showed that less than 34% of target cells contained aspartate or glycine, but over 50% contained GABA or glutamate, consistent with a high proportion of amacrine cell targets. These data suggest that regenerating photoreceptors may prefer nonappropriate target cell types, however, the transmitter content of most target cells (GABA, glutamate) is similar to that of cells postsynaptic to photoreceptors in the intact retina.

SYNAPTIC COMPETITION BETWEEN SUPERNORMAL AND NORMAL SENSORY NEURONS IN THE COCKROACH IS MEDITATED THROUGH A CHANGE IN QUANTAL CONTENT AND NOT QUANTAL SIZE. M.A. Song* and J.M. Blagburn, Insitute of Neurobiology, University of Puerto Rico School of Medicine, San Juan, Puerto Rico 00925

The cens of the first instar cockroach (Periplaneta americana) bear two filiform hairs each, one lateral (L) and one medial (M), which are innervated by individual sensory neurons. These sensory neurons project to the CNS, where they synapse with giant interneurons (GIs) that project the perirenal ganglionic. Mutant strains have been previously discovered which have an extra L hair or one on both cens. The sensory neurons that innervates the extra hair, known as Spaces Injector (SN), projects to the same GIs as the normal L hair innervated neurons for these synaptic targets. The size of the GI3-EPSP in GI3 is reduced in the presence of SIN. We wanted to determine whether this reduction in ESPP is a reduction of the postsynaptic current (giant akin). This led us to ask if the ESPP amplitude is the result of pre- or post-synaptic changes. Intracellular recordings from GIs in a modified open bath showed no increased size of L hair, were made in normal cockroach saline (or 5 mM CaCl2/10 mM MgCl2) (low Ca), 

SYNAPTOGENSESIS AND SYNAPTIC PLASTICITY IN DISSOCIATED CHICK CEREBRAL NEURONS. T. Taguchi, K. Kojima, K. Kuroi, A. Ishida, E. Suzuki, O. Fujito, K. Nishikawa, K. Ohishi, T. Kato, and K. Izumi, Dept. of Biology, Kyoto University, Osaka, Japan

We used electrophysiological methods to study the synapse between two cell types: two different types of amacrine cells. We wanted to know whether synapses between two different types of amacrine cells are able to make a functional connection. We found that the synapse between two different types of amacrine cells can make a functional connection.

INTERACTIONS BETWEEN PHOTODAMAGED AND INTACT TERMINALS AT POLYNEURONALLY INNervATED FROG NEUROMUSCULAR JUNCTIONS. J. Thum, I. Tak, and A.A. Horny, Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90009

Our goal is to study how changes induced in one motor nerve terminal can affect another in polyneuronally innervated neuromuscular junctions in frog. This approach is important in understanding the role and nature of competition during synaptic elimination in neuromuscular junctions. Reinnervated sarcolemma of Rana pipiens has a substantial percentage of polyneuronally innervated junctions 8 weeks after nerve crush. Our previous in vivo observations indicated that under normal conditions such junctions are functionally stable over a period of several weeks which makes artificially induced changes easily detectable in this system. To induce changes in a small portion of a terminal in vivo we used the fluorescent dye 4-Di-2-Asp and low-light video microscopy was used for repeat in vivo observations of exposed and intact terminals. In cases where reexposure of the receptors, no changes were detected in intact terminals in the same neuromuscular junction a week after photodamage. We are not yet able to make repeated exposures of identified neuromuscular junctions over longer periods of time after selective photodamage. Our study also includes an electrophysiological examination of changes in postsynaptic potentials of one input in a doubly innervated junction effects the other input.

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450.15

A HIGH NUMBER OF GABA SYNAPSES TARGETING DISTAL PORTIONS OF CORTICAL NEURONS IS LOST AFTER PERIPHERAL DEPRIVATION. J. Michéa*, K. Crozier and C. Crevier, Dept Pathology and Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, (Qué) CANADA.

The numerical density (N), and proportion (%) of GABA synapses on dendritic spines, shafts, somata, and initial segments of the axon was estimated in the somatosensory barrel fields of 5 rats having their vibrissae from the right face continuously removed from birth. For the cortical dendrites, no significant differences in the N, and % of GABA synapses on these post synaptic elements were found in the barrel field cortex contralateral and ipsilateral to the deprivation. About 19.7 million GABA synapses per mm² of tissue were devoted to spines (representing 3.8% of all contacts) on spines 2.55 million were on dendritic shafts (38.1%), 5.6 million on somata (71.0%) and less than 0.01 million on the initial segments of the axon (100%). In layer IV however, sensory deprivation induced a drop from 55.6 in the ipsi cortex to only 11.9 million/mm² of GABA synapses on spines in the contra cortex (a 4.7 times loss: p<0.0001) and from 82.2 to 33.8 million/mm² on shafts (p<0.005). Only 2.2% and 29.1% of all contacts on spines and shafts respectively, were GABA in the contra cortex as compared to as many as 93.9% and 49.1% in the ipsi layer IV. We conclude that the population of GABA synapses located on distal portions of layer IV neurons is the most plastic of all populations of cortical synapses. (Supported by MRC, FRSQ, and FCAR).

450.17

Development of iterated structures in the NMDA receptor deficient mice. Yung Li¹, Reha S. Erurumlu², Chong Chen, Sonal Jhaveri² & Susumu Tonegawa HMMI at the Ctr. for Cancer Res., ¹Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA 02139.

Sensory pathways of the brain generally develop from crudely wired networks to precisely organized system and in some cases to iterated structures such as stellar dendrite columns and barrel fields in the cerebral cortex. Several studies have implicated neural activity-dependent mechanisms including NMDA receptors, in this refinement process. We applied the gene targeting in mouse embryonic stem cells to the NMDAR1 gene and created a mutant mouse which lacks functional NMDA receptors. We analyzed the formation of iterated structures in the mutant mice. The development of glomeruli in the olfactory bulb of mutant mice is compared to that of wild type animals. The number of glomeruli in the mutant mice is the same as their normal littermates, suggesting that the development of glomeruli is not dependent on NMDA receptor activation. In contrast, whisker-related patterns in the trigeminal nuclei (barrellets) failed to develop as judged by both cytochrome oxidase staining and immunohistochemistry, although patterning, initial targeting and crude topographic projection of trigeminal axons in the brainstem are unaffected. In addition, in the absence of functional NMDA receptors, the synaptic transmission from trigeminal ganglion to the second order neurons in the brainstem trigeminal complex is functional in mutant mice. These results suggest that NMDA receptor and/or activity is essential for the development of somatosensory maps in the mammalian brain. Funded by HHMI and the Shonogi Inst. for Medical Science (ST) and NS27678 (SJ).

450.19

HOMONYMOUS NEUROMUSCULAR PREPARATION USING THE CAMPENOT CHAMBER. A. Parfitt, E.A. Neale*, C.C. Fitzgerald and P.G. Nelson: Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

We have shown that mouse ventral horn motor neurons grown on mouse cortical astrocytes, in the side wells of Campenot chambers that have been scaled onto laminin-coated plastic culture dishes, will extend axons underneath the chamber barrier into the center compartment, there to make functional contact with muscle striated muscle fibers. These fibers will contract in response to electrical field stimulation across one or both of the Campenot barriers. Most field-induced contractions could be blocked by rhodaminated- bungarotoxin, which was used to label post synaptic cholinergic receptors, suggesting that they were neurally mediated and not the result of direct electrical stimulation of muscle fibers which had grown into or under the Campenot barrier. Neurites that were immunoreactive for CHAT could also be found in association with muscle fibers that had contracted in response to electric field stimulation. Cells and their processes in the side wells of the Camponot chamber could be stained with Fast DII/DIO. Intracellular diffusion of these dyes along the neurites that passed under the chamber barrier permits visualization, and will facilitate the differential characterization, of the neurite fields in the center compartment. Preliminary experimental data include evidence for activity-dependent synapse elimination.

450.16

SELECTIVE DECREASE IN THE NUMBER OF GABA SYNAPSES IN LAYER IV AFTER SENSORY DEPRIVATION. J. Michéa*, K. Crozier and C. Crevier, Dept Pathology and Centre de Recherche en Sciences Neurologiques, Université de Montréal, (Qué) CANADA.

The effects of modified peripheral activity at cortical level were estimated by quantifying the overall and GABA synapatic populations in the somatosensory barrel field cortex of 5 rats whose whiskers on the right face were continuously removed after birth.

The numerical density of GABA synapses was found to decrease more than twice (p<0.005) in layer IV contralateral to the deprivation (63 million/mm²) compared to the ipsilateral layer IV (145 million/mm²). Accordingly, GABA synapses represented only 9.4% of the overall synaptic population in the contralateral versus 18.7% in the ipsilateral layer IV. At the same time, GABA synapses in the deprived layer IV were bigger (0.48 μm in the contralateral layer IV vs. 0.32 μm in the ipsilateral layer IV; p<0.05). No significant changes in the numerical density, proportion or size of GABA synapses were detected in any other cortical layer. No differences in these parameters were found for the total synaptic population either.

As the firing activity and receptive field properties of cortical neurons are under the control of GABA, such a selective reduction in the number of GABA synapses in layer IV, the major recipient of thalamocortical inputs, would undoubtedly lead to profound functional alterations in the entire circuitry of the deprived somatosensory cortex.

Supported by MRC, FCAR and FRSQ, Canada.

450.18


The adult pattern of axonal connections from the eye to the brain arises during development by refinement of a roughly ordered pattern of connections in chick. One characteristic of the early pattern of connections is a transient ipsilateral retinotectal projection. Previous studies showed that administration of arginine analogs prevented the loss of this transient ipsilateral projection. The most likely interpretation of this result is that the drug inhibited nitric oxide (NO) synthesis in the visual system, and that NO is somehow involved in the refinement process. The present study examined whether administration of an arginine analog actually reduces NO synthesis and identified the most likely site of action. L-NOARG was applied to the chorioallantoic membrane of embryos. Controls received saline. On E13, the middle of the period of refinement, nitric oxide synthase (NOS) activity was assayed by measuring the conversion of arginine to citrulline in homogenates of retina and tectum. In normal embryos, NO activity was detected in the tectum but not in the retina. This was consistent with the histochemical detection of NOS in these tissues during the period of refinement. NO activity in the tectum was inhibited in the L-NOARG treated group but not in the saline treated group. The effect of L-NOARG was dose-related. There was a correlation between the level of NO synthesis inhibition and the degree of preservation of the ipsilateral projection in the tectum. These results suggest that NO serves as a messenger from the tectal cells back to the retinal axons and that it is involved in the developmental refinement of the visual projection. (Supported by EY0371).

450.20


Thrombin, at nM concentrations, has been implicated in the process of activity-dependent synapse reduction (ADSR) in a mouse neuromuscular junction preparation in vitro (Liu et al., Soc. Neurosci Abs, this volume). This is based on the block of ADSR by nM concentrations of hirudin, a highly specific thrombin inhibitor. Also exogenous thrombin (~10 nM) causes synapse loss which is blocked by hirudin. We have, therefore, investigated the regulation of thrombin release from muscle cells. We compared the levels of thrombin in media conditioned by muscle cells treated with tetrodotoxin (TTX, 1 μM, to block activity) or acetylcholine (ACH, 10 μM, to activate the cells). Significantly more thrombin was released by the ACh treated cells (1056 nM ± 0.03 nM; S.D., n=18 with ACh vs .027 ± 0.02 nM, t=15 with TTX). (The medium with 5% horse serum contained 0.066 nM thrombin). We hypothesize that increased thrombin secretion produced by muscle activation could contribute to ADSR.
451.1

OVEREXPRESSION OF NGF IN TRANSGENIC MICE INDUCES NEUROTROPHIC FACTORS AND ALTERS PRIMARY SENSORY NEURONS. B.M. Davis1*, D.M. Katz2*, K.B. Srogovsky and K.M. Albers3. 1Depts. of Anatomy & Neurobiology, and 2Pathology, Univ. of Kentucky College of Medicine, Lexington, KY 40536; *Dept. of Neurosciences, Biomedical Research Unit, University Hospital of Cleveland, Cleveland, OH 44106.

Peripheral nerve crush induces novel projections from sympathetic neurons to skin in transgenic mice that overexpress NGF in the skin. Specifically, a large proportion of primary sensory neurons in NGF transgenic mice were innervated by tyrosine hydroxylase (TH)-positive pericellular (PC) nerve fibers that were seen only rarely in controls. Removal of the superior cervical ganglion abolished TH-immunoreactive arborizations in the ipsilateral trigeminal ganglion confirming the axonic effects. A two-site ELISA revealed that transgenic ganglia contained a ten fold increase in NGF peptide. Reverse transcriptase PCR analysis showed no apparent expression of the transgene in sensory ganglia suggesting that the additional NGF was derived from increased NGF expression in the skin. These results indicate that NGF induces novel sympathetic projections to sensory neurons, and supports the hypothesis that increased NGF expression is a critical link in the development of sympathetic hyperalgiesia. Supported by AR-40873 (KMA), NS-31826 (BMD), HL-42131 (DMK) and IBN-9221136 (KBS).

451.2


Neurotrophins and their high affinity binding proteins (HABP) have been identified as novel neurotrophic factors (BDNF, neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), are a family of homologous proteins that affect neuronal cell survival and support neuronal growth and differentiation. During development, neurotrophins are made by neuronal target tissues such as skin and the decline in their expression levels correlates with the onset of naturally occurring neuronal cell death. To examine the role of neurotrophins in the development of the peripheral nervous system, we isolated transgenic mice that express fusion gene constructs containing a human keratin (K14) gene promoter linked to the versant mouse neurotrophin cDNAs. Transgene expression begins in the basal cell layer of epidermis around embryonic day 14 (E14), coinciding with the normal decline in neurotrophin levels. Previously we have isolated and characterized mice containing a K14-NGF transgene. In this study, mice expressing a K14-BDNF transgene have been isolated. BDNF is normally expressed in neuronal tissues of both the central and peripheral nervous system and is lost in neuronal tissues such as heart, lung and skin. By overexpressing BDNF in the epidermis, its role in development and in the adult may be elucidated. Three lines of mice have been isolated that express the K14- BDNF transgene as shown by both Southern and Northern hybridizations. Mice appear normal in size and health. Initial histological examination of the skin and sensory ganglia show no major alterations in these structures. Supported by NS-31826 (BMD) and AR-40873 (KMA).

451.3


Neurotrophins and their high affinity binding proteins (HABP) have been identified as novel neurotrophic factor, (BDNF, neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), exhibit wide overlapping effects to skin in sensory ganglia suggesting that the additional NGF was derived from increased NGF expression in the skin. These results indicate that NGF induces novel sympathetic projections to sensory neurons, and supports the hypothesis that increased NGF expression is a critical link in the development of sympathetic hyperalgiesia. Supported by AR-40873 (KMA), NS-31826 (BMD), HL-42131 (DMK) and IBN-9221136 (KBS).

451.4

NGF OVEREXPRESSIN in EPIDERMIS Disrupts SYMpathetic INNERVATION OF SWEAT GLANDS AND DIFFERENTIALLY ALters TRANSPReSSOR Molecules. G. Gudary*, B. M. Davis2, S. C. Landis3 and K. M. Albers4. 2Dept. of Neurosci., Case Western Reserve Univ., Cleveland OH 44106, 3Dept. of Anatomy and Neurobiol. and 4Dept. of Path. Univ. of Kentucky College of Medicine, Lexington KY 40536.

We examined sympathetic innervation in transgenic mice carrying multiple copies of the NGF gene driven by an epidermal keratin promoter. In adult wild-type mice, cholinergic and noradrenergic sympathetic fibers and peptidergic sensory fibers innervate the distal tail. Acetylcholinesterase (ACHE) and vasoactive intestinal peptide (VIP) sympathetic axons were associated with sweat glands while catecholaminergic fibers innervated blood vessels. The NGF overexpression was restricted to tissues in the pad area. Calcitonin gene-related peptide (CGRP) and substance P (SP) sensory fibers traversed the pad and terminated at the interface between the epidermis and dermis. While the timing of innervation in footpads did not appear to be affected in transgenic mice, segregation of sympathetic and sensory innervation was altered. A dense plexus of ACHE and catecholaminergic fibers was found beneath the epidermis and VIP fibers extended into the epidermis. The plexus was absent from footpads of mice treated with the adrenergic neurotoxin 6-OHDA, indicating its sympathetic origin. This same region was hyperinnervated by CGRP and SP sensory fibers. In addition, sweat gland innervation was disrupted. While some ganglia were innervated by ACHE and VIP fibers, most lacked innervation. Despite the sparse gland innervation, sympathetic responsiveness appeared normal. Our findings suggest that over-expression of NGF in skin interfered with the normal segregation of sensory and sympathetic fibers. Further, the ecotopic sympathetic fibers displayed an abnormal neurotransmitter phenotype, expressing both catecholamines and cholinergic properties.

451.5


Brain derived neurotrophic factor is a member of the family of related molecules, which includes NGF, NT-3 and NT-4/5. Neurotrophic effects on neuron survival and differentiation in vivo and in vitro suggest important roles for these molecules on essential developmental processes such as programmed cell death and determination of neuronal phenotype. As part of our efforts to clarify the in vivo role(s) of the neurotrophin genes, using gene targeting methodologies, we have generated mice in which most of the sequence coding for BDNF was deleted and replaced with a neomycin resistance gene. Homozygous mutant mice (+/--) are reduced in size compared to the wild type mice and die within the first three postnatal weeks. Analysis of DRG cell numbers and size indicates that many neurons die in the (-/-) animals. Heterozygous (+/-) were viable but consistently intermediate in size. This problem was examined in the function of three primaryafferents innervating skin in adult (+/+) mice using an in vivo recording technique (Kohlaugeg et al. Soc Neurosci., this meeting). Briefly, single primary afferents were recorded in the saphenous nerve using standard trac- ted fiber techniques. All cutaneous primary afferent types were encountered and characterized in wild type and (+/--) animals (Ai, Al and C-fibers), however, only one type was severely affected in the (-/-) fibers. Slowly adapting, non myelinating fibers, conducting in the A8 fiber range, had very elevated mechanical thresholds. These initial results suggest that BDNF is critically important for the regulation of this afferent type. It will be important to see if total absence of BDNF in (+/--) animals has more widespread effects on the functional properties of sensory neurons.

451.6

TRANSGENIC MICE OVEREXpressing BDNF IN NEURAL STEM CELLS. Torkel Falkenberg1,2*, Thomas Ringstedt2,3, Erik Nilsson2, Urban Lendahl2, Ron McKay3, Hakan Persson2 and Carlos F. Bates1. 1Molecular Neurobiology, Medical Biochemistry and Biophysics, 2Department of Women and Child health, 3Department of Bioclinical Science, Bioclinical Biology, Cell and Molecular Biology, Bioclinical Institute of Physiology, Karolinska Institute, Stockholm, Sweden, 4Molecular Biology, NIH, Bethesda, MD, USA.

Neural stem cells in the neuroepithelium of the neural tube are transiently present during embryonic life and later differentiate to form neurons and glial cells. The neural stem cell state is accompanied by expression of the intermediate filament nestin. Regulatory regions controlling the nestin expression in the stem cells have recently been characterized in transgenic mice using lacZ as a reporter gene. We have analyzed the effects of overexpression of the neurotrophic factor BDNF (brain derived neurotrophic factor) using transgenic mice harboring BDNF under the control of regulatory regions of the nestin gene so the coding portion of the BDNF gene. This DNA construct was injected into fertilized mouse eggs with the attempt to generate transgenic mouse lines. With this particular construct we have observed a very low frequency of postnatal transgenic mice (6% of all offspring were transgenic mice). However, one of the founders did not express BDNF from the exogenous gene, while another one did not pass the gene further to its offspring. Thezera suggest that BDNF may be a significant regulator of neural stem cells, which may be critical for the maintenance of the transgenic embryos. Further work is aimed at analyzing phenotypes in transgenic embryos at different developmental stages.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
451.8 VISCERAL SENSORY NEURONS ARE SEVERELY DEPLETED IN MICE LACKING FUNCTIONAL ctkb PROTEIN TYROSINE KINASE RECEPTORS. J.T. Erickson*, J.J. Simones*, M. Babacik* and D.M. Kanz*. Departments of Neurosciences and Medicine, Case Western Reserve University School of Medicine, Cleveland, OH 44106 and Department of Molecular Biology, Bristol-Myers Squibb, Princeton, NJ 08543.

451.10 STRUCTURE AND TRANSGENIC EXPRESSION OF RAT NEUROTYPHON-4 GENE. P. Metsinis, S. Salin, Y. Hurj*** and T. Timmusk. Lab. of Molecular Neurobiology, Karolinska Institute and *Dept of Clinical Neuroscience, Karolinska Hospital, Stockholm, Sweden.

Neuropathic pain and related disorders are a major health problem affecting millions of people. However, the underlying mechanisms of pain due to nerve damage remain largely unclear. Recent studies have revealed that the degeneration of sensory neurons is not only a secondary event in neuropathic pain, but also plays a key role in the initiation and maintenance of pain. In this study, we investigated the structural and transgenic expression of neurontrophin-4 (NT-4) gene in various tissues to understand its role in the development of neuropathic pain.

Neurontrophin-4 (NT-4) expression is very low in the adult organism, while higher levels are present during embryogenesis. Developmentally regulated expression of NT-4 is thought to contribute to the hypothesis of its target derived action on the trigeminal ganglion. To study the regulation of the NT-4 gene and to evaluate the possible roles of this neuropot in developing and an adult system we have characterized rat NT-4 gene. A cDNA library was made from P1 testis, where a high expression level was previously detected. Screening of this library resulted in 10 CDNA clones that were used to screen a rat genomic library. 10 overlapping genomic clones were isolated that covered 45 kb of the NT-4 locus. Mapping of the intron-exon structure revealed two upstream noncoding exons separated by an 800 bp intron. Transient expression analyses with bacterial chloramphenicol acetyl transferase reporter gene suggests an important role of intronic sequences in regulating the promoter activity. Rat NT-4 gene fragment containing upstream exons, promoter region and coding exon was introduced into transgenic mice. The expression pattern of the transgene recapitulates the endogenous gene expression pattern and regulation. Transgenic mice with high level of NT-4 expression have altered levels of other members of neurotrophin family in distinct brain regions.
451.13 EVALUATION OF GROWTH FACTOR EFFECTS ON MESENCEPHALIC DOPAMINERGIC NEURONS USING A FOS-LACZ TRANSGENIC MOUSE LINE J. Engels* and K. Schilling, Dept. Anatomy and Cell Biology, University of Ulm, 89069 Ulm, FRG.

One of the initial effects of many growth factors is the rapid and transient induction of the immediate early gene c-fos in their target cells. In the present study, we have exploited this feature to probe the cellular target(s) of growth factors previously observed to support survival of cultured dopaminergic neurons. Specifically, we used low density cultures established from the dissociated mesencephalon of E14 fos-lacZ transgenic mice (generously provided by J.J. Morgan, Roche Institute, Nutley, N.J.) to monitor c-fos expression in immunocytochemically identified cellular phenotypes. NT-3 (10 ng/ml, 3 hrs), bFGF (25 ng/ml) or a combination of aFGF (50 ng/ml) and heparin (2.5 U/ml) induced transient expression in subpopulations of tyrosine hydroxylase-immunoreactive dopaminergic neurons as well as non-dopaminergic neurons and glial cells. The effects of these growth factors on dopaminergic neurons were not affected by cellular plating density or by eliminating synaptic communication. In contrast, TGFα (50 ng/ml) and PDGF-BB (50 ng/ml) induced transient expression exclusively in glial cells but not in dopaminergic neurons.

These studies identify mesencephalic dopaminergic neurons as the primary target of NT-3 and FGF and further demonstrate that sensitivity for these growth factors is restricted to distinct subsets of dopaminergic neurons. Finally, our results suggest that the known survival-promoting effects of TGFα and PDGF-BB on dopaminergic neurons are mediated through mesencephalic glia.

451.14 NEUROTROPHIC FACTORS AND CEREBRAL VESSELS OF TRANSPLANTED MOTOR NEURONS DERIVED FROM THE E14-FOS-LACZ TRANSGENIC MOUSE.


A number of workers have made use of transplantation and retroviral-mediated gene insertion using a variety of monoclones in order to immortalise neural precursor cells. This study used a conditionally immortalised hippocampal cell population derived from the E14-Fos-LacZ transgenic mouse. The mouse possesses an established integrated copy of the early region of the large tumour antigen from the temperature sensitive gene allowing immortality to be controlled by the permissive temperature of 33°C, the non-permissive temperature being 39.5°C. We examined the effects of several growth factors including basic fibroblast growth factor (bFGF), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), the tyrosines were p75-neurotrophin and ciliary neurotrophin factor (CNTF) on E14 transgenic mouse hippocampal progenitor cells.

Initial results showed that NGF produced an elevation in both survival and proliferation in the hippocampal cells with maximal effects produced at a concentration of 10ng/ml. NGF produced minimal increase in cell number, but did however induce differentiation when kept at 36.5°C. The other 2 neurotrophins (BDNF and NT-3) as well as CNTF produced interesting results which will be shown in the poster. Cells treated with FGF in general were lower in number and longevity at the higher concentrations.

Partially supported by the Welcome Trust.

451.15 ROLE OF ESTROGEN IN AFFECTING INCREASED ALCOHOL INTAKE IN TRANSGENIC TGα-MICE. Leena Hikmat-Clark*, Lorenzo Hikmat-Clark, Lombardi Cancer Research Center, Georgetown University, Washington, DC 20007.

Results with transgenic CD1 mice that overexpress transforming growth factor α (TGα-M) suggest that this growth factor alters non-reproductive sex-related differences in behavior. Specifically, male TGα-M mice exhibit a feminization of locomotor activity, immobility in the swim test, and sodium preference. These effects may be mediated through an interaction between TGα-M and estrogen. The TGα-M mice exhibit elevated plasma 17β-estradiol (E2) levels. Castration reverses the feminized behavioral patterns in the male TGα-M mice. The present study investigated the effect of orchectomy, E2 and the antiestrogen tamoxifen on voluntary alcohol consumption in transgenic TGα-M mice. Both the male and female transgenics consumed more 5% alcohol (expressed as a percentage of total fluid intake) than the non-transgenic mice (P<1.39=8.8, p<0.005). Orchietomy did not have a significant effect on alcohol intake. In non-transgenic CD1 male mice, treatment with pellets releasing E2 increased and treatment with tamoxifen reduced alcohol intake, when compared with placebo-treated mice (HSD=25.2, p<0.001). In ovariecetomized females, E2 reduced alcohol intake (H4)=12.6, p<0.006). These data suggest that overexpression of TGα increases alcohol consumption in both sexes. Estrogen also increases alcohol intake in non-transgenic male mice, and this is reduced by the antiestrogen tamoxifen. However, since orchietomy at adulthood did not reverse the increased alcohol intake patterns in the transgenic mice, the effects of TGα on alcohol consumption may be mediated through other pathways than elevated E2. Alternatively, the effects of E2 occurred during the critical period of sexual differentiation.


We are interested in examining mechanisms regulating estrogen actions during neuronal differentiation in the central nervous system. Our research has focused on one possible mechanism, the developmental interations of nerve growth factor (NGF) and estrogen receptors. Using a combination of steroid autoradiography, immunohistochemistry and in situ hybridization methodologies, we have shown that estrogen target neurons of the developing forebrain express the NGF receptors, p75NGFR (the pan-neurotrophin receptor) and rn4 (the specific tyrosine kinase receptor). Moreover, NGF and estrogen reciprocally regulate their receptors in P12 cells, a neurotrophin-responsive cell line. In this study, we examined the regulation of estrogen binding by NGF in the developing forebrain using organotypic explant cultures maintained in roller tube assemblies. Explant cultures of the postnatal day 2 rat cerebral cortex and basal forebrain were maintained 8 days in vitro, in the presence or absence of NGF (100 ng/ml). Our data indicate that estrogen receptors and receptor mRNA are expressed in these cultures, in patterns similar to those observed in vivo. Using a modified nuclear exchange assay to measure specific, intr-nuclear estrogen (3H-moxestrol) binding, we found that NGF alters nuclear estrogen binding in a regionally-specific manner. We are currently examining mechanisms underlying regionally-specific NGF regulation of estrogen binding. (Supported by grants from the NIH, NIMH, NSF, AHA and an ADAMHA-RA to C.D.-A.)

451.17 NEUROTROPHIC FACTORS FAIL TO PREVENT CAstration-INDUCED REGRESSIVE CHANGES IN AN ANDROGEN-SENSITIVE RAT SPINAL NUCLeUS. M.C. Clark-Phelps*, T.R. Nett, and D.R. Sengelaub1

Program in Neural Science, Indiana University, Bloomington, IN 47405: Cephalon, Inc., West Chester, PA 19380.

Motoneurons of the rat spinal nucleus of the bulbocavernosus (SNB) are sensitive to androgens. Castration of adults significantly reduces SNB dendritic length, soma size, and target muscle weight, and these changes can be prevented or reversed with androgen treatment. Using compounds demonstrated to have trophic effects on motoneurons in several models, we assessed maintenance of muscle weight and motoneuron morphology of the androgen-sensitive SNB system following castration in adulthood.

Adult male rats (Sprague-Dawley) at approximately 90 days of age were either castrated or left intact. Castrated rats received daily subcutaneous injections over the SNB target muscles of either IGF-I (1 mg/kg), CNTF (1 µg), or vehicle alone for six weeks. Following treatment, SNB target muscles (bulbocavernosus and levator ani; BCLA) were weighed and motoneuron morphology was visualized histochemically after retrograde labeling with a cholera toxin-TRH conjugate. Castration significantly reduced SNB soma size and BCLA muscle weight relative to intact males, and treatment with either IGF-I or CNTF had no effect. Thus, unlike androgen, IGF-I and CNTF failed to prevent castration-induced regressive changes in these aspects of SNB morphology, suggesting that the androgen-dependent trophic effects observed in this system are not mediated by these compounds in adulthood. (Supported by Cephalon)

The hippocampus is a primary site of neurotrophin mRNA expression in both developing and adult rats. Neonatal sympathetic ganglia transplanted into adult rat hippocampus grow into the dentate gyrus and CA3 regions, which display NGF-like staining. The discrete boundaries of this staining suggest the occurrence of NGF, or a related neurotrophin, firmly bound to hippocampal structure, supplemented by a pool of soluble NGF. NGF-like immunoreactive immunostaining in the hippocampus was blocked by the monoclonal antibody directed against NGF (WNI-2), suggesting that NGF is bound to hippocampal structure. In addition, we have developed an immunocytochemical method utilizing a monoclonal antibody directed against NGF immunoreactivity. Our results suggest that NGF is primarily bound to hippocampal structure.


We have used purified, well-characterized granule cell cultures (Gao et al. 1991) for this study. Neurons 6705-715 to examine the possible effects of the neurotrophins on four different stages of cerebellar granule cell neurogenesis. The four stages included neuronal proliferation, maturation, neuronal differentiation, and maintenance. None of the neurotrophins stimulated proliferation of the granule cell precursors or rescued granule cells that were eliminated by neuronal insults. However, neurotrophin-4/5 (NT-4/5) and BDNF, but not neurotrophin-3 (NT-3) and neurotrophin-2 (NT-2), promoted neurite extension and survival of differentiated cerebellar granule cells. Moreover, NT-4/5 and BDNF enhanced neurite extension by unjured granule cells, which were rescued from various types of cell death in the hippocampus. These findings suggest that NT-4/5 and BDNF promote the development and maintenance of differentiated granule cells, which are downstream to the neuron. In addition, the effects of these factors were seen with the combination of NT-4/5 and BDNF. The neuron-promoting and survival effects of NT-4/5 and BDNF could be completely blocked by the specific tyrosine kinase inhibitor K-252a. Thus, these two neurotrophins activate the same receptor tkβ for signal transduction.
452.7

INVOVLEMENT OF NEUROTROPHINS IN THE PHENOTYPIC SPECIFICATION OF CHICK CUTANEOUS AFFERENTS: G.R. Lenzi1*, M. Koltenborg, K.V.Troyka, and Y.A. Barde
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Neurotrophins may influence the phenotypic fate of sensory neurons during development. Here we have used an electrophysiological recording technique to study the functional properties of sensory neurons whose access to neurotrophins was manipulated in vivo. Monoclonal antibodies (anti-NGF and anti-NT3) were delivered by hybridoma cells placed on the choroidallastic membrane at E1, while NT3 was delivered at E17 and staining with rhodamine was made from cutaneous sensory neurons as described (Koltenborg et al. Soc. Neurosci. this meeting). After anti-NGF treatment fibers conducting in the C-fiber range (<1 m/s) were reduced by 50%, while none of the fibers responded to noxious heat, although all had mechanical thresholds similar to those in normal chicks (i.e., they were C-M fibers). In contrast to untreated controls animals where nearly 50% of the fibers responded to noxious heat (C-MB), the functional properties of presumptive A-fibers appeared normal (conduction velocity >2 m/s). This is consistent with the idea that only C-MB fibers in the chick depend on NGF for survival. After NT3 treatment the conduction velocity range of recorded afferents was indistinguishable from controls. A-fibers also appeared unaffected by the treatment in terms of mechanical threshold. However, amongst the C-fiber population all the fibers studied (n=12) had unusually low mechanical thresholds, and none responded to noxious heat. Comparison of the NT3 treated C-fibers to control A-fibers revealed that their mechanical thresholds were very similar. Anti-NT3 treatment did not appear to affect the physiology of C-fiber afferents. These results indicate that the availability of neurotrophins can selectively affect the functional properties of developing sensory neurons in vivo.

452.8

SINGLE UNIT RECORDINGS OF CHICK CUTANEOUS SENSORY NEURONS IN VITRO AND IN DRUGS: G.R. Lenzi1, K.V. Troyka, and Y.A. Barde

Chick sensory neurons have been commonly used as a model system to study the effects of neurotrophins on neuronal survival in vitro. As these DRG neurons are highly sensitive to NGF deprivation, we have chosen to characterize functionally different types during late embryonic development and early post-hatching. The cutaneous teres majoris mediates pain sensation which was dissected out together with the skin of its innervation territory and placed in an organ bath. Single unit recordings were obtained using conventional techniques and units were analyzed with controlled mechanical, thermal and chemical stimuli. A total of 91 neurons conducting between 0.5-10 m/s were studied. Units displayed no ongoing and mechanical thresholds (von Frey hairs) ranged from 1-64 mN. Of 44 units conducting <1 m/s many had probably non-sense function, as 16/34 were excited by noxious heat (thresholds 35-47°C) and >430 by noxious cold. Application of 10mM bradykinin, 5-HT, histamine, PGE2 activated 14/27 units all of which were also thermosensitive. Following a chemical stimulation 1/14 units displayed lowered heat thresholds and stronger suprathreshold responses indicating sensitization. Heat-insensitive fibers did not respond to chemical stimulation. During the ongoing developmental interval mechanical thresholds tended to increase. At all ages units conducting >2 m/s (presumptive A-fibers) could be classified on the basis of a slowly or rapidly responses to innocuous mechanical stimuli. The results indicate that the cutaneous-nervous in vitro preparation is suitable for the study of functional properties of diverse cutaneous sensory neurons both during embryogenesis and the early post-hatching period.

452.9

INFLUENCE OF NEUROTROPHINS ON THE DEVELOPMENT OF PRIMARY AFFERENT PROJECTIONS IN THE CHICK SPINAL CORD: A.L. Edie*, G.B. Lewin and Y.A. Barde
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Primary afferent projections in the chick can be studied using the lipophilic tracer DiI. In the present experiments we have examined the influence of neurotrophin treatment or deprivation with monoclonal antibodies to NT3 and NGF. NT-3 was delivered by NT-3 secretion A-293 cells placed on the choroidallastic membrane at E15, whilst antibodies were delivered by hybridoma cells. At E12 or E14 treated and control embryos were fixed and DiI was injected into the L5 DRG, the L5 spinal nerve or into an identified muscle or skin. All afferent fibers or primary afferents have long ranging projections outside their segment of entry and have collaterals at least 6 segments below their segment of entry (Ekemalm and Comp Neuror, in press). In contrast, L5 fibers in anti-NT3 treated embryos projected primarily to their segment of entry. Furthermore, axons projects ventrally to the motoneuron pool although those labeled only retrogradely were seen innervating all dorsal horn laminae. Interestingly, sensory neurons and their collaterals in the dorsal horn were labeled from muscle nerves, but few collaterals were seen in the ventral horn. After NT-3 application intersegmental projections appeared normal, although there appeared to be fewer collaterals in L2-3 segment of entry. This may be related to a change in the phenotype of sensory neurons treated with NT-3 (Lewin et al. Soc. Neurosci Abs this volume). Finally, animals treated with anti-NGF showed little projection to laminae, which is consistent the idea that C-M fibers are killed by this treatment. The results suggest that primary afferent projections may be modulated by the availability of neurotrophins.

452.10

POSTNATAL AUDITORY NEURONES DEPEND UPON BRAIN DELINEATED NEUROTROPHIC FACTOR FOR SURVIVAL: M. LeBleu*, J.E. Boudreau and R. Lefebvre
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Both aR/3 and aR/4 immunolocalized over neurons while aR/2a was associated with the supporting cells of the postnatal spiral ganglion. Using in situ RT-PCR of postnatal cochlea tissue sections, we identified products for NGF, BDNF and the NGF isoform in this tissue. Organotypic explants of postnatal organ of Corti were cultured in the presence of either antisense or sense nucleotides (5mM) for NGF, BDEN or NT3. ELISA testing showed a 90% reduction in NGF protein levels in response to NGF-AS oligo. Evaluation of the effects of neurotrophin antisense treatment in explants was accomplished by confocal microscopy of antiGlut1 immunostained whole mount. BDNF-AS treatment resulted in neuronal cell death. NT-3-AS treatment affected synaptic contacts, and downregulation of NGF destabilized perisome platinic findings demonstrate that the neurotrophins studied play separate, unique but interrelated roles in postnatal organ of Corti explants. (Supported by grants from NIH, DOD0488 to TRV, HS, NSF of Belgium to FPL and GM).

452.11

HIGH-AFFINITY NEUROTROPHIN RECEPTOR EXPRESSION IN THE CAT RETINA DURING NORMAL DEVELOPMENT AND FOLLOWING NEONATAL VISUAL CORTEX DAMAGE: V.R. King, J.T. Xue, and P.D. Spear
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We recently showed that normal 1-4 week-old kittens that displayed pericellular labeling of the high-affinity nerve growth factor receptor (p140NGF) in the retinal ganglion cell layer (GCL). In addition, kittens that receive a visual cortex lesion at E14 show normal 1-4 week-old kittens. Soma and dendrite labeling indicated that many of these cells are ganglion cells. Kitten that received V3 damage on day of birth had greater pericellular labeling in the hemisphere projecting to the damaged hemisphere than in the hemisphere projecting to the intact hemisphere. This difference was seen in kittens that survived from 3 days to 8 weeks, but not for 12 weeks. It also was seen in kittens at 12 weeks of age, but not for 26 weeks. Truncated k1a labeling was light and limited to the nerve fiber layer. The p140NGF receptors may be involved in retinal development and plasticity. In addition, both nerve growth factor, which binds preferentially to k1a, and BDNF may affect the retina via Müller cells.

452.12

NORADRENERGIC NEURONS IN THE LUCUS COEULEUS OF BIRDS EXPRESS TRKA AND TRP AND RESPOND TO NGF: G.S. von Bardeleben, A. Schober, Y. Kadonaga, R. Williams, and M. Bothwell
Dept. of Physiology and Biophysics, University of Washington, Seattle, WA 98195 & Dept. of Developmental Biology, Biomedical Center, Uppsala University, Uppsala, Sweden.

The noradrenergic neurons in the chicken locus coeruleus express the neurotrophin receptor p75 (see von Bardeleben et al. Brain Res. 599:320-479, 1992). To determine which neurotrophin may regulate these neurons, expression of trk receptors was examined by in situ hybridization. A subpopulation of neurons in the locus coeruleus expresses trkA receptor at 9 and 18 days of incubation (El and E18). To test if these neurons can bind and internalize NGF, retrograde transport of radio-iodinated NGF was examined. Neurons in the locus coeruleus of E15 chick embryos accumulate 1:125 NGF after injections into the basal forebrain. The retrograde transport of radio-iodinated NGF is restricted to the noradrenergic neuronal population as evidenced by double-labeling with an antibody to dopamine-beta-hydroxylase (DBH). To test if these neurons respond to NGF, explants cultures and dissociated neurons were treated with or without NGF. NGF did not significantly improve neurite outgrowth from E15 explants or the survival and morphology of DBH-labeled coeruleus neurons that were dissociated at E12 and labeled with DBH antibody two days later. In vivo, the site of DBH-labeled coeruleus neurons was visualized by injections of NGF into the telencephalon of E19-20 embryos, but NGF did not rescue these neurons from toxin-induced cell death after injections of 6-hydroxydopamine. These data suggest that noradrenergic neurons may play a role in the development and physiology of the avian locus coeruleus. The data confirm major differences in the occurrence of neurotrophic factors in birds and mammals with regard to trophic regulation of presumptive homologous neuronal populations. Supported by grants from NIH (HD 29717, HS 30305), DFG (F98V-1/1) and the Medical Faculty of Uppsala University.

Several neurotrophic factors enhance motoneuronal survival in vitro and in vivo. The most potent of those reported to date, the neurotrophins BDNF, NT-3 and NT-4/5, act by binding to specific membrane receptors shared by all other neurotrophins. We tested recombinant hGDNF on purified motoneurones from E14 rat spinal cord. After 5 days in culture, GDNF maintained nearly all (>90%) motoneurones that initially developed; similar efficacy was obtained with mouse response curves revealed that GDNF was 20- to 50-fold more potent than the neurotrophins; half-maximal survival was achieved at 0.01-0.1 ng/ml.

Using RT-PCR, GDNF mRNA was detected in E15 rat limb bud, cultured neonatal Schwann cells and embryonic myotubes; levels in skin were considerably lower. In situ hybridization on sections of E14.5 rat embryos showed signal over some muscle masses and some developing nerve tracts; labeling was essentially absent from the spinal cord and skin.

Taken together, these findings suggest that GDNF plays an important role in early motoneuronal development. Its actions may be complementary to those of the neurotrophins (and perhaps other factors) both during development and in therapeutic approaches to human motoneurone disease.


Systemic NGF injections in fetal rats preserve excess ganglionic cells and interspersed TGF-β related patterns in the hindbrain (Henderson et al., J. Neurosci. 14, 94; primary afferents in the hindbrain also lack somatotopic patterns and collaterals and do not have ephaxtor axons. Because these findings suggest mechanisms by which the periphery makes CNS patterns, it is important to understand how NGF alters pattern formation. Oligo NGF preserve (or induce) a transient ephaxtor projection to whisker-like surfaces that lessens disparity in the innervation densities of the whisker follicles and intervening skin, wherein lesioning the "digitized" nature of the resultant CNS map. A related idea is that NGF accelerates development of a late-arriving innervation of inter-whisker-like, leading to the same CNS effect. These hypotheses are tested by systematically injecting rats with NGF on embryonic days 15 and 18, sacrificing at birth, confirming that CNS pattern were absent, and examining the myotaxic pad innervation with PFG 9.5 and RTF7 immunofluorescence. Comparable infraorbital nerve fascicles in NGF- treated neonates were larger and contained more labeled axons than in normal neonates, although the innervation of whisker follicles seemed comparable in both distribution and maturation. However, preliminary observations indicate that the innervation of the inter-digit ephaxtor is more mature in the NGF-treated cases. These data support both of the above hypotheses and suggest that neurotrophins control pattern formation by orchestrating innervation density patterns in the whiskerpad during a critical period in development. NIH DE07374, NS17763, NS24679.
545.2  Evidence for the role of DARP (Dopamine-Releasing Protein) during the development of rat dopaminergic neurons and the adrenal gland.  
D.A. Linnerord, Y.D. Ralphs, Department of Molecular and Integrative Physiology, University of Illinois at Urbana Champaign 61801 
Recent work from this laboratory suggests a role for DARP during development. DARP is a glycoprotein that is secreted from the cortical striatum in vivo (Brain Res. 463: 335) and has been immunocytochemically located in the adrenal gland (Neuroendocrinology 58: 444) and in close association with catecholaminergic neurons in the rat CNS (Neuroendocrinology, in press). In addition, intracranial administration of an anti-DARP monoclonal antibody (DARP mAb) in embryonic day 10 rats failed to alter the pattern in response to a dose-dependent manner (Mol. Cell. Neurosci. 2: 410). In this study, ELISA analysis indicated that normal DARP concentrations were maintained in the brain extracts from E17 brains that were two to three times higher than those reported for P5 rats. These findings add to the evidence that DARP is present in the embryonic and early postnatal rat brain and may play a role in the pre- and postnatal development of dopaminergic neurons of the mesencephalon and the postnatal development of the adrenal gland.

NEUROTROPHIC FACTORS: BIOLOGICAL EFFECTS X

545.1 RECOMBINANT HUMAN INSULIN-LIKE GROWTH FACTOR--I (hIGF-I) PREVENTS CISPLATIN-INDUCED NEUROPATHY  
Injection of the endogenous excitotoxic quinolinic acid into the rat striatum produces a lesion which is neurochemically similar to that seen in Huntingdon's disease. We examined whether neurotrophin-3 (NT-3), a member of the neurotrophin family of growth factors, is capable of reducing the damage caused by quinolinic acid in the rat striatum. NT-3 (0.15 or 0.5 μg/kg) was infused via osmotic pump into the brain hemisphere for 12 days, beginning immediately after injection of quinolinic acid (225 nmol in 1 μl PBS). Control rats received PBS infusions following quinolinic acid injection. Rats were sacrificed on day 12 and both the lesioned and intact striatum dissected for measurement of choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) activities, neurochemical markers for cholinergic and GABAergic neurons, respectively. Quinolinic acid injection depleted GABA activity by 13% in the intact, uninjected side; NT-3 infusion at a dose of 0.15 mg/kg attenuated the effect of quinolinic acid, with ChAT activity on the lesioned side reduced by only 12% (p<0.05), while NT-3 at a dose of 0.5 mg/kg did not have a significant effect on the depletion of GAD activity. These findings suggest a possible therapeutic role for neurotrophic factors in reducing or reversing neuronal damage in Huntingdon's disease.

545.2 NEUROTROPHIN-3 REDUCES DEPLETION OF STRIATAL CHAT AND GAD ACTIVITIES FOLLOWING QUINOLINIC ACID LESION  
Injection of the endogenous excitotoxic quinolinic acid into the rat striatum produces a lesion which is neurochemically similar to that seen in Huntingdon's disease. We examined whether neurotrophin-3 (NT-3), a member of the neurotrophin family of growth factors, is capable of reducing the damage caused by quinolinic acid in the rat striatum. NT-3 (0.15 or 0.5 μg/kg) was infused via osmotic pump into the brain hemisphere for 12 days, beginning immediately after injection of quinolinic acid (225 nmol in 1 μl PBS). Control rats received PBS infusions following quinolinic acid injection. Rats were sacrificed on day 12 and both the lesioned and intact striatum dissected for measurement of choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) activities, neurochemical markers for cholinergic and GABAergic neurons, respectively. Quinolinic acid injection depleted GABA activity by 13% in the intact, uninjected side; NT-3 infusion at a dose of 0.15 mg/kg attenuated the effect of quinolinic acid, with ChAT activity on the lesioned side reduced by only 12% (p<0.05), while NT-3 at a dose of 0.5 mg/kg did not have a significant effect on the depletion of GAD activity. These findings suggest a possible therapeutic role for neurotrophic factors in reducing or reversing neuronal damage in Huntingdon's disease.

545.3 NEUROTROPHIN-4/5 AND TRANSFORMING GROWTH FACTOR--A PARTIALLY PROTECT STRIATAL CALBINDIN-CONTAINING NEURONS AFTER QUINOLINIC ACID LESION  
T. Akef*, V. Usunoff and E. Helft, Andrus Gerontology Center and Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089. 
Lesioning of the parasympathetic preganglionic cholinergic neurons by the excitotoxin quinolinic acid (QA) results in the degeneration of medium spiny neurons that contain γ-aminobutyric acid (GABA). Neurotrophic factors have been shown to rescue degenerating cortical pyramidal neurons in vitro and in vivo. We have tested the efficacy of neurotrophin-4/5 (NT-4/5) and transforming growth factor--α (TGF--α) on the QA lesioned striatum. Conclusions were made for administration to rats lesioned acutely with QA. Caudate rats received a single unilateral intrastriatal injection of QA (150 μg). Treated rats were injected either with NT-4/5 (0.6 μg), TGF--α (0.8 μg) or both (0.6 μg NT-4/5 and 0.8 μg TGF--α) into the QAx-injected side. Control rats received saline injections. The animals were sacrificed 7 days after injection. 

545.4 FAILURE OF CONTINUOUS INTRATHecal NEC INFUSION TO PREVENT TOXIC CHEMICAL-INDUCED DISTAL AXONOPATHY DESPITE REPEATING THE INJURY-INDUCED UPREGULATION OF C-FUN EXPRESSION IN DRG NEURONS  
B.C. Golub*, T. Stenson-Dickerson and D.R. Antin, Center for Research on Occupational & Environmental Toxicology (CROET) and Department of Cell Biology & Anatomy, Oregon Health Sciences University, Portland, OR 97239. 
Clinical trials of NFL for the treatment of peripheral neuropathies have begun. However, direct (morphological) evidence that NFL prevents axonal degeneration is lacking. In the present study, we asked whether supplementing the cell body's supply of neurotrophic factors by intrathecally perfusing NFL prevents the development of distal axonal degeneration in a proangiogenic model of distal axon degeneration. 3,4-Dimethyl-5-2-  
hexanone (DMDH), a potent derivative of the gamma-diketone, was given daily intrathecally to naive rats for 2 weeks. Rats were perfused with 5% paraformaldehyde at 7 days. The axons were etched in 20% ammonium hydroxide to reveal the axons' Neurophilic factors may provide protection for subpopulations of striatal cells. 

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BNF PROTECTS CULTURED DENTATE GRANULE CELLS AGAINST HYPOGLYCEMIC DAMAGE

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We have previously shown (Lindvall et al., 1991, 1992, 89, 648-652) that and 10 min of insulin-induced hypoglycemic coma led to a marked increase of BNF and NGF mRNA levels in dentate granule cells. The aim of this study was to explore whether brain-derived neurotrophic factor (BNF) in insulin-deprived cultures of rat dentate granule cells subjected to a hypoglycemic insult. Dentate gyrus was dissected from rat pups (P5-6), dissociated and cells were plated on chamber slides at 37°C in 1% CO2 and with 95% humidity. After 24 h the serum-containing medium was switched to serum-free N2 medium. The hypoglycemic insult was induced after 7 days in vitro by glucose deprivation. The duration of hypoglycemia was 3h and cultures were fixed and processed for MAP-2 immuno-cytchemistry immediately thereafter. In control cultures 40% of cells was stained. Glucose deprivation for 15h caused severe neuronal loss (about 70%). BNF added 24h before or 6h after onset of hypoglycemia completely protected granule cells against the insult-induced damage. Nerve growth factor (NGF) had similar effects. These findings support the hypothesis that the rapid up-regulation of BNF and NGF mRNAs in dentate granule cells after brief periods of hypoglycemic coma and other insults is a local protective mechanism.

453.5
A QUANTITATIVE ANALYSIS OF ASTROGLIAL AND MICROGLIAL CELL REACTIONS IN PRIMARY SENSORY SPINAL TRUNK TRANSECTION: FOLLOWING SCARCE NERVE INJURY AND TREATMENT WITH NERVE GROWTH FACTOR IN THE ADULT RAT

Eriksson S., Eriksson K. E., Persson M., Portera-Cailliau C., and Halldin C.*
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Previous quantitative immunohistochemical studies have shown that the microglial cell reaction in primary sensory projection areas after peripheral nerve injury follows a very rapid exponential growth phase which is succeeded by a constant exponential growth phase; the peak level is reached after 5-10 days and is sustained for more than 70 days after injury (Eriksson et al., Exp. Brain Res., 1993; 96-127). Further, it has been shown that following peripheral nerve injury, astrocytes are activated via reactive microglial cells (SVV et al., J. Neurosci., 1993; 13: 273-303). Using image analysis we have examined quantitatively the temporal appearance of the astroglial reaction to scarrc nerve injury as revealed by the area of glial fibrillary acidic protein (GFAP) immunoreactivity in the dentate granule nucleus. Concomitantly, the temporal appearance of GFAP-mRNA profiles were observed by in situ hybridization. Astroglial cells in the dorsal horn and the granule nucleus were seen to follow similar growth and decline curves with an increase to two days and a maximum to two-three weeks following scarrc nerve transection with nerve growth factor (NGF) during four weeks following scarrc nerve transection did not result in any different glial response, as investigated with our quantitative methods. These results indicate, that even though promoting dorsal root ganglion (DRG) cell survival, NGF does not seem to have any influence on the glial cell reaction neither directly nor indirectly through the innuited DRG-neurons. The microglial cell reaction therefore does not seem to be triggered by the target loss of the sensory neurons.

453.6
CHRONIC NGF TREATMENT RESTORES HIPPOCAMPAL MUSCARINIC, BUT NOT NICOTINIC RECEPTOR FUNCTION FOLLOWING FIMBRIAL TRANSECTION

P. A. Landshø, D.M. Araya and F. Hed, Andersson Neurology Center, University of Southern California, Los Angeles, CA 90089-0191.

The effects of chronic NGF administration (1 mg qod for 21 days) on hippocampal muscarinic and nicotinic receptor densities and functions were determined in adult rats with partial fimbrial transections. The first study, the distribution of cholinergic receptors in the hippocampus was determined using autoradiography. Neither fimbrial transections nor NGF treatment altered the density of muscarinic M1 (H-pirenzepine) or M2 (H-2AF-DX) binding sites in the hippocampus. In addition, neither the lesion nor NGF treatment altered the distribution or density of nicotinic [3H]-diethylaminoethylazabenzimidazole-2(1H)tetrahydroazepine (TPH) uptake in the hippocampus. Second, we determined the effects of NGF treatment on muscarinic receptor-mediated second messenger production in rats with fimbrial transections. In lesioned rats, oxotremorine i.p. increased IP3 synthesis by 81% compared to the contralateral unlesioned side. This lesion-induced supersensitivity of M1 muscarinic receptor function was prevented by chronic NGF treatment. Third, we determined whether nicotine receptor functions using the rat fimbrial transection model and nicotinic-induced uptake of [3H]-tetraphenylphosphonium (TTPP) into hippocampal synaptosomes as a marker. In the unlesioned control hippocampus, nicotine produced a characteristic 35% reduction of [3H]-TTPP uptake into synaptosomes. However, in rats with partial fimbrial transections there was a loss of nicotine-induced alterations of [3H]-TTPP uptake into synaptosomes. Chronic NGF treatment did not affect nicotine-induced [3H]-TTPP uptake, nor did it reverse the lesion induced loss of presynaptic hippocampal cholinergic functions involved in ACh turnover (Lapchak, Exp. Neurol. 124, 16-20, 1993) translate into an enhanced function of postsynaptic muscarinic M1 receptor function. However, chronic NGF treatment does not attenuate the lesion-induced loss of presynaptic nicotinic autoreceptor function.

453.7
THE MICROGlia/MACROGliE RESPONSE FOLLOWING NGF INJECTION INTO THE HIPPOCAMPUS

M. Knott, D. Elegua, K. Andrews, M. Sipes, & A. Avendaño, Department of Neurological Surgery, University of Washington School of Medicine & Seattle VA Medical Center, Seattle, WA.

The response of microglia and macrophages during waldrogen degeneration of axons is remarkably different in the peripheral PNS and central CNS. Nervous system of adult mammals (Soffin et al., 1993; Perry et al., 1987). In the PNS, blood monocytes rapidly infiltrate degenerating nerve to clear and neural debris, in contrast, the recruitment of monocytes and response of microglia with clearance of cellular debris is much slower in the CNS. The mechanisms underlying the differential recruitment of microglia and macrophages are unclear. Neurone Growth Factor (NGF) has been shown to be a possible chemotactic factor for leukocyte recruitment (Boyle et al., 1985). The low affinity (NGF) and high affinity (NGF) receptor have been detected in a number of mouse and rat isolates (Lin et al., 1990, and Yan and Johnson, 1990; Morgan et al., 1989). In addition, a linear arrangement of macrophages was found immediately adjacent to NGF treated but not untreated trophic implants injected into rat spinal cords (Houle, 1992). NGF may therefore play a role in modulating the response of microglia and macrophages in the CNS. To test this hypothesis, we performed local needle injections of NGF, vehicle (2% bovine serum albumin), into needle injections into rat spinal cords either with or without an injection of an adjacent dorsal cord (12). We employed the ED1 immuno-microscopy which normally stains peripheral ral macrophages and monocytes but not CNS microglia. Immunohistochemistry was performed upon paraflxed eneved paraffine sections of spinal cords. Following simple transection of a dorsal root, we found ED1 labeled cells infiltrating the PNS portion of the cut dorsal root after a delay of days that stopped abruptly at the PNS/CNS interface and did not extend into the spinal cord for up to 3 weeks. Needle injection into the spinal cord induced the local appearance of ED1 staining cells. The number of cells and volume of tissue staining with ED1 was greater following injections of NGF, significantly less with injections of vehicle alone, and smallest following needle injection alone. Spinal cord injections of NGF combined with an adjacent dorsal root resulted in ED1 labeled cells extending across the PNS/CNS interface of the cut dorsal root towards the injection site. These results suggest that NGF may activate microglia to express ED1 and/or induce the migration of peripheral ED1 monocytes/microphages into the CNS. Supported by NH and VA Funds.
453.11


Retinal ganglion cell (RGC) survival can be enhanced by the intracranial administration of BDNF or NT-4 (Mansour-Robay et al., PNAS 91: 1652-1656, 1994. Chau et al., submitted). Is there still a role for NT-3 on RGC survival and regeneration? Intracranial injections of NT-3 (provided by Regeneron Pharmaceuticals Inc.) had only a marginal effect on the overall survival of gap43-expressing RGCs, consistent with the observation that immunocytochemistry for TrkC, the high affinity receptor for NT-3, revealed staining in only 5% of RGCs. However, in comparison to the more neurotrophic, NT-3 increased intraretinal axonal growth, as revealed by staining with RT91, a monoclonal antibody to the heavy neurofilament subunit.

The effect of NT-3 on the expression of GAP43 mRNA was then investigated by in situ hybridization. Two weeks after axotomy, approximately 10% of surviving RGCs expressed high levels of GAP43 mRNA. After NT-3 treatment, the incidence of these GAP43-expressing cells increased to 30% of surviving RGCs. Furthermore, GAP43 mRNA levels within these cells doubled, compared to operated controls. These findings suggest that NT-3 has a significant effect in increasing GAP43 expression and on axonal growth of a discrete population of RGCs.

453.13

EFFECT OF NT-4/S IN AXOTOMIZED RAT FACIAL MOTOR NEURONES. Karl Fernandes, Annie Beaudet and Wolfram Teissieg. Dept. of Physiology, University of Ottawa, Ottawa, Canada.

We have previously shown that axotomy of facial motoneurons induces the expression of GAP-43 and Tau-1 tubulin mRNAs while the expression of neurofilament mRNA is decreased. In addition, we report here that the mRNA expression for AChE is reduced by about 35% consistent with the general downregulation of neurotransmitter related mRNAs after axotomy. In the present study we have tested the role of a presumed target derived neurotrophin, NT-4/S, in the regulation of these changes in gene expression. The facial nerve of male adult Sprague Dawley rats was transected at the stylomastoid foramen and the proximal nerve stump attached to the lumen of a silastic tubing. The latter was connected to a chronic minipump which delivered 1 ul with 125 ng (low dose) or 500 ng (high dose) NT-4 per hour over a period of 7 days. The contralateral proximal nerve stump received vehicle only. In situ hybridization revealed that application of NT-4/S sustained the expression of ACNE mRNA at normal levels (202 % of vehicle, n=4) and further stimulated the expression of GAP-43 (211 % of vehicle, n=4) and Tau-1 tubulin (170 % of vehicle, n=4) mRNA. The expression of neurofilament-M (NFM) mRNA was increased to 150% of vehicle (n=4), but was only significantly different from vehicle in 1 of 4 animals. NFM expression was still far below a normal level of expression, thus, not normalized by NT-4 treatment.

These data show that NT-4/S enhances regeneration associated gene (GAP-43, Tau-1 tubulin) expression and might be useful to stimulate peripheral nerve regeneration. Supported by MRC of Canada.

453.15

EFFECT OF CNTF DELIVERY METHODS TO RESCUE FACIAL MOTONEURONES FROM INJURY-INDUCED CELL DEATH. E.A. Tarin, V. Pedrun, A. Mercier, J. Hammang, E. Batzios, A.O. Zurn, P. Astrid, Lausanne University Medical School, Switzerland; CytoTherapeutics, Providence, RI.

Ciliary Neurotrophic Factor (CNTF) has been shown to increase the survival of motoneurons in vitro and in vivo. For potential human application, the mode of delivery needs to be investigated. In the present study, local application of CNTF on the transected facial nerve of neonatal rats was compared to systemic delivery through a murine recombinant (rhCNTF) and to transplantation of genetically engineered cells. Baby hamster kidney cells (BHK) were transfected with a pMNJ vector containing either the gene for mouse CNTF (mCNTF) or human CNTF (hCNTF) and were encapsulated in polypropylene fibers to prevent tumor formation and immune rejection. Facial nerves of neonatal (P2) rats were transected and CNTF was delivered by direct application of CNTF on the nerve stump using gelatin impregnated with mCNTF (0.25mg/ml); ii) by repeated subcutaneous injections of rhCNTF (1mg/kg) 3 times a week; or iii) by subcutaneous implantation of 1x10^6 encapsulated BHK cells releasing either mCNTF or hCNTF. Control animals received either bovine serum albumin or the parent BHK cell line. One week post-injury, the number of surviving motoneurons on the lesioned side was compared to the non-lesioned side. All three methods of CNTF application significantly improved motoneuron survival with the encapsulated method appearing to be the most efficient.

Mode of delivery Control CNTF treated p values

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<tr>
<td>rhCNTF capsule</td>
<td>11% 40%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>mCNTF capsule</td>
<td>11% 38%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>repeated injection</td>
<td>14% 39%</td>
<td>p=0.005</td>
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<tr>
<td>gelatin application</td>
<td>18% 31%</td>
<td>p&lt;0.005</td>
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Encapsulated transduced cells offer a mean for the continuous slow release of trophic factors, as well as the potential for intrathalamic delivery.

453.12

ROLE OF NT-3 IN INTACT & INJURED PRIMARY SENSORY NEURONS. K.A. Gratton, P. Friedman & L. Lin. 2 R.N. Lindeman 4 1 Dept. of Anatomy, University of Saskatchewan, Canada; 5700 W. Regeneron Pharmaceuticals Inc., Tarrytown, NY 10951.

The hypothesis that NT-3 plays a role in maintaining the differentiated state of responsive trkC-expressing neurons and may be responsible for reversing changes observed after injury, in vivo was tested. Adult rat right sciatic nerves were proximally cut. 14d after injury NT-3 was intrathecally infused for an additional 7d in half of the rats. Crystal sections of intact and injured DRG with and without NT-3 infusion were processed for in situ hybridization to detect mRNAs encoding trkC and peptides a-CGRP and NPY. Preliminary results indicate that in intact neurons a-CGRP and trkC are abundantly and heterogenously expressed, whereas few if any neurons express detectable NPY mRNA. Two weeks after injury levels of a-CGRP and trkC mRNA are dramatically reduced, while, many neurons now express abundant NPY mRNA. Infusion of NT-3 counteracted injury-induced decreases in trkC mRNA and was also effective in upregulating expression of a-CGRP in many neurons, but the percentage of neurons appeared smaller when compared to injured ganglia infused with NGF. Finally, NT-3 infusion resulted in reduced expression of NPY mRNA in injured neurons, suggesting a role for NT-3 in gene suppression in intact neurons. The ability of exogenous NT-3 to regulate expression of its receptor trkC and differentially regulate peptide expression supports a role for it in maintaining aspects of the differentiated state of adult primary sensory neurons. Canadian MRC supported.

453.14

NT-3 AND BDNF PREVENT AXOTOMY INDUCED DEATH OF CORTICOSPINAL NEURONS. Klaus M.G. Giehl* and Wolfram Teissieg. Dept. of Physiology, University of Ottawa, Ottawa, Canada.

A major problem of regeneration in the CNS is atrophy and cell death of injured neurons. We have quantified the death of corticospinal neurons (CSN) induced by axotomy at the level of the internal capsule and tested the effect of survival promoting factors in this system. In vivo hybridization combined with retrograde tracing revealed that CSN express mRNAs for both trkA and trkB but not trkC receptors. This provided the rationale to test the survival effect of Neurotrophins in this model. Again, the CSN were positively identified by spinal cord injection of Fast Blue and a second spinal cord injection of Rhodamine Dextran was applied after internal capsule lesion to control for the area of CSN extended in anterior posterior direction over 5.6mm. A mean of 49.5% (n=4) of the axotomized CSN died within 7 days after injury. Continuous application of saline (0.9% rat serum albumin [vehicle]) via osmotic minipump over 7 days reduced this cell death to 30.4% (n=8). Application of NGF (500ng/ul) resulted in further reduction to 24.2% (n=4), however this was not significantly different from the vehicle. The application of NT-3 (50ng/ul) or BDNF (500ng/ul) prevented the axotomy induced death and only 3.7% (NT-3, n=7) and 10.2% (BDNF, n=4) of the CSN died within the first week after axotomy. These findings are the first demonstration for survival factors for CSN in vivo and may be important for traumatic brain injury and in neurdenerative disorders which involve death of CSN like amyotrophic lateral sclerosis. Supported by MRC and Network of Centres of Excellence for Neural Regeneration and Functional Recovery (Canada).

453.16


Brain-derived neurotrophic factor (BDNF) promotes the survival, differentiation and maintenance of motoneurons. We compared the effects of different BDNF doses on the rescue of axotomized sciatic and facial motoneurons in neonatal rats. Application of BDNF directly to the central nerve stump significantly enhanced motoneuron survival. Additional BDNF supplied with intermittent subcutaneous injections (1 mg/kg, 3 day intervals) yielded a further small survival increase; however, injecting BDNF daily markedly reduced the motoneuron rescue at both 1 week and 2 weeks post-injury. These results, corroborated by findings in motoneuron primary cultures, show that a dose-dependent reversal of BDNF-mediated motoneuron rescue may occur both in vitro and in vivo.
453.17

Conditionally immortalized neural progenitors were infected with a retrovirus coding for mnGfP, and clones were isolated and analyzed for NGF production. Among them, one clonal line of NGF-negative/mnGfP- cells was characterized in vitro for their ability to produce NGF and for the expression of the retroviral vector, finding no differences between the proliferative and growth arrested cultures (permissive and non-permissive temperatures). After grafting to the septum, striatum or nucleus basalis in young animal, the cells survive well and migrate away from the implantation site, and neurite formation is observed. NGF production was studied in dissected pieces of nc basalis tissue 4 and 10 weeks after grafting. RT-PCR revealed moderate but stable expression of the transgene, and a bouquet for NGF demonstrated antigen and NGF-like activity. Immunohistochemical staining for NGF showed high neurotrophic activity on PC12 cells (the hippocampal tissue). Cells grafted to the substantia nigra induced a consistent 40% hypertrophy of the cholinergic cell bodies (1, 4 and 10 weeks post-grafting). When grafted to the septum in rats with fornix lesion, 30% of the cholinergic cells on the lesion side was rescued from acetycholine induced death. Thus, there seems to be a good correlation between cell survival, expression of the transgene, NGF production and cellular effects observed.

We next looked for behavioral effects of the NGF cells in vivo by grafting into the medial septum and/or nc basalis of memory impaired aged animals. In the Morris water maze test, there was a progressive improvement of their performance (2 and 4 weeks post-grafting) up to the point when they were not different from the control, non-impaired animals. Histological analyses revealed surviving grafted cells, as well as a clear hypertrophic effect in the host cholinergic neurons, that showed the same size as in young rats. An increased expression of NGF-like neurotrophic activity from grafted brains will also be presented.

453.19

Subcutaneous injection of acidic fibroblast growth factor (aFGF) into senescence accelerated mice (SAM-R/1) at one per week was started at 3 weeks after birth and continued for 9 months. When tested by passive avoidance and Morris water maze tests, the mice learning and tasking performances were deteriorated in the control group, while those of the aFGF-treated group did not. At the end of the 9 months, brain and striatal tissues were stained immunohistochemically using anti-cholin acetyltransferase (CAT) antibody. The number of cholinergic neurons in the septum was decreased by 15% in the control group and was no change in the aFGF-group treated, as compared to SAM-R/1, a reference strain of SAM-R/8. The activity of CAT in individual cholinergic neurons in the MS also decreased significantly in the control group. Results suggest that aFGF has neurotrophic effects on cholinergic neurons in the MS of SAM-R/8 and ameliorate learning and memory disorder in both tasks tested.

454.1
Effects of BDNF in animal models of parkinson's disease. C.W. Shults, C. Shline, and C.A. Alkon. +VA Med. Ctr., San Diego, CA 92161; Dept. of Neuroscience, Univ. Cal., San Diego; +Regional Research & Ttreatment Center, NY.

Groups of 8 naive rats received 3 unilateral, intrastriatal injections of either BDNF or cytocrome c (22.5 µg/injection) on consecutive days. of the injection or control treatment. On the second day, the animals received an intrastriatal injection of 25 µg of 6-hydroxydopamine hydrobromide (6-OHDA). The animals were then tested weekly for amphetamine and apomorphine-induced rotations in left and right sides. The BDNF treated animals had fewer apomorphine-induced contraversive and amphetamine-induced ipsiversive rotations than did the cytocrome c treated group. At the fourth test week, the BDNF treated group had 0.8 ± 0.4 (mean ± SEM) apomorphine-induced and 0.7 ± 0.8 amphetamine-induced rotations/min, and the cytocrome c treated group had 3.1 ± 1.0 apomorphine-induced and 2.7 ± 0.6 amphetamine-induced rotations/min.

Two groups of rats with partial unilateral lesions of the mesostriatal dopaminergic system, which had been matched for apomorphine-induced rotations, were injected with either BDNF or cytocrome c (22.5 µg/injection) into the partially denervated striatum over 2 weeks. After the sixth injection the BDNF-treated group had significantly fewer apomorphine-induced rotations (2.6 ± 0.4, n=10) than the cytocrome c treated group (5.4 ± 1.0, n=11).

454.2
Effects of BDNF-producing fibroblasts on substantia nigra dopaminergic neurons in vivo. W. B. Galvin, I. K. Tatton, M. F. Peal, D. M. Franl, and H. O. Breckfield, and D. Factor. +Neurogeneregulatory Laboratory, McLean Hospital, Belmont, MA 02178; 2Molecular Neuromeration Unit, Neurosurgery and Neurology Services, Massachusetts General Hospital, Boston, MA 02114; 3School of Medicine, University of Massachusetts, Worcester, MA 01655.

We have previously demonstrated that brain-derived neurotrophic factor (BDNF) is able to protect against dopaminergic (DA) cell loss in the substantia nigra pars compacta (SNc) following striatal infusion of the mitochondrial complex I inhibitor MPTP in rats. To investigate the effects of BDNF on SNc neuronal function in vivo, we performed the following experiments: BDNF was delivered for 4 weeks using microinjection pipettes inserted to the SNc. Half of the implanted rats received unilateral striatal infusion of MPP+ one week after grafting. For the non-lesioned animals, tissue was processed for tyrosine hydroxylase (TH) immunohistochemistry or for biochemical analysis 1-2 weeks following implantation. Lesioned animals were sacrificed 2 weeks after implantation, and the effects of BDNF on the generation of free radicals using salicylic acid-trapping as well as thiobarbituric acid assay were performed. Initial biochemical observations reveal an increase in DA content in SNc neurons associated with BDNF administration. Preliminary assessment of the effects of BDNF on cellular morphology indicate there is no generalized effect on TH+ cell size in the SN. Further experiments aimed at determining the effect of BDNF on the metabolic and oxidative states of DA neurons in intact and MPP+ lesioned rats are in progress.
545.3

Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor for dopaminergic neuronal cells, but the cell type that degenerates in Parkinson's disease(PD) To examine the potential BDNF gene therapy for PD, astrocytes transduced with a BDNF cDNA or alkaline phosphatase (AP) cDNA (control) and prelabeled with Dil were grafted into the right rat striatum 15 days after partial lesioning of the right substantia nigra with 6-hydroxydopamine. Prior to grafting, BDNF mRNA was expressed by BDNF but not AP astrocytes. At 32 days, BDNF astrocytes, but not AP astrocytes, attenuated amphetamine-induced rotation by 45% (p<0.05). Aporphamine-induced rotation was not significantly changed in either group. At 42 days, Dil labeled astrocytes were present in the graft site, however, BDNF mRNA positive cells were not detected with in situ hybridization. Analysis of the density of dopamine transporter (DAT) immunoreactive fibers showed no effect of BDNF astrocytes on the area occupied by TH-positive fibers in the lesioned striatum. These findings suggest that BDNF gene therapy ameliorates Parkinsonian symptoms through a mechanism that does not involve regeneration or sprouting from DA neurons. Furthermore, expression of BDNF from this retroviral construct appears to be down-regulated after a critical period. The efficacy of this method will be addressed before this method can be utilized for long-term delivery of biologically produced BDNF. (Supported by Merck-Kasirsky Trust & AG00926)

545.4

Cholinergic neurons which have been genetically modified to secrete brain-derived neurotrophic factor (BDNF) are effective in enhancing the neuronal survival and neurite extension of embryonic rat dopaminergic neurons in vitro. Rotated mesencephalic tissue transplants containing such embryos were harvested, dissociated, and cultured in a serum-containing medium. Shortly thereafter, three BDNF oligodendrocyte clones (A1, B6, and B12) and the non-BDNF secreting parental line (N1) were individually plated with rMT and rMT and cultured in a 37°C incubator. The coculture medium was changed to a serum-free medium about 24 hours and placed back into the incubator for 4 days. The number of dopaminergic neurons as well as the number of neurite extensions per neuron were then quantified in 7-8 replicates using tyrosine hydroxylase (TH) immunocytochemistry. An mean increase in TH-positive neurons of 5.9%, 41.0%, and 10.5% was observed in cocultures with clones A1, B6, and B12, respectively when compared to rMT alone or rMT cocultured with N201 oligodendrocytes. Thirty-four% of TH-immunoreactive neurons exhibited 3 or more processes when cultured alone or with N201. In contrast, 60%, 80% and 84% of TH-immunoreactive neurons exhibited 3 or more processes when cocultured with the A1, B6, and B12 clones, respectively. BDNF-secreting oligodendrocytes enhance the viability and neuronal differentiation of embryonic dopaminergic ventral mesencephalic neurons suggesting that BDNF oligodendrocytes might enhance the structural and functional consequences of fetal nigral grafts in animal models of Parkinson's Disease.

546.4

The delivery of neurotrophic factors to the CNS poses a major obstacle to the treatment of neurodegenerative disorders such as Parkinson's disease. We have developed a polymer encapsulation technology that provides a means for the delivery of BDNF (generally provided by Regeneron) for a prolonged period of time. Several different types of chitosans, algatanes and co-polymers with various proportions of poly-L-lysine were used. Size distribution profiles were determined by an image analysis system and surface characteristics were assessed by electron microscopy. The mean diameter of microspheres with BDNF was 1.2-1.6 μm whereas spheres with genetically transformed cells secreting BDNF (provided by X.O. Breakefield and M.P. Shotton) were much larger (300-500 μm). The total content of BDNF as well as the amount released per day were determined by dot blot assays. The results from the release kinetics demonstrate that longterm secretion (30-60 days) of BDNF is achieved by chitosan microspheres. Alginate spheres provided only relatively short term release (2-7 days) and the type of macromolecules encapsulated with BDNF significantly influenced the rate of BDNF delivery. Neuron survival and neurite growth in cultures of rat retinal ganglion cells, DRG, and midbrain floor were supported by microencapsulated BDNF indicating biological stability of BDNF. BDNF-secreting cells survived for different periods of times depending on cell density within spheres, sphere sizes, and properties of the semipermeable membranes. These model systems can also be used for delivery of other trophic factors such as CNTF and GDNF. These studies provide a basis for future comparisons of microencapsulated genetically engineered cells and agents used in the treatments of neurodegenerative disorders.

546.5
REGULATION OF PLATELET-DERIVED GROWTH FACTOR (PDGF) AFTER NEURONAL LESIONS AND EFFECTS OF PDGF ON DOPAMINERGIC AND STRIATAL NEURONS IN VITRO. K. Piao 1, K. Fujii 2, Y. Nakajima 1, A. Ichiba 1, T. Tsukamoto 1 and O. Ito 1*, 1Dept. of Pharmacology, graduates School, University of Tokyo, Bunkyo-ku, Tokyo, Japan and 2Dept. of Neuroscience, Kurume Medical School, Kurume, Fukuoka, Japan.

We have previously demonstrated an increased expression of platelet-derived growth factor (PDGF) around intrasplenial implants of fetal dopamine (DA)-rich mesencephalic tissue in a rat model of Parkinson's disease. PDGF-BB but not PDGF-AA promotes survival and neurite outgrowth from rat and human DA neurons in vitro and influences expression of c-fos and tyrosine hydroxylase mRNA in mesencephalic cells. These effects could be directly mediated via PDGF beta receptors expressed on the mesencephalic DA neurons. The objective of the present study was to further characterize the changes of PDGF expression following intrasplenial implants of DA-rich mesencephalic tissue and to analyze the effects of PDGF-AA and -BB on striatal DARPP-32-positive neurons in vitro. Immunohistochemistry, PCR and primary cell culture techniques were used. Following an isotonic acid lesion to the rat striatum (model of Parkinson's disease), a strong increase in the expression of PDGF was observed in astrocytes in the striatal tissue. Also increased PDGF receptor expression was induced in the lesioned area. In cell cultures of fetal striatal PDGF-BB but not PDGF-AA promoted the survival of DARPP-32-positive neurons. Also neurite outgrowth from these striatal cells was significantly more pronounced in the presence of PDGF-BB. These findings indicate that PDGF could be of importance for the survival and function of CNS neurons after lesions and in neurodegenerative processes.

546.8
NEUROTROPHIC FACTORS AND CELL DEATH OF A CELL LINE DERIVED FROM RAT VENTRAL MENENCEPHALON. H. Takashima*, Department of Neurology, Kawata National Hospital, Kawatana, Nagasaki 859-36, Japan.

Basic fibroblast growth factor (bFGF) has neurotrophic effects on dopaminergic neurons. Recently, reduced concentration of bFGF in substantia nigra in the Parkinson's disease brain has been reported. Reduction of this factor might be related to the cell death of dopaminergic neuron in Parkinson's disease. In this study, the effects of neurotrophic factors on the differentiation and cell death of a neuronal cell line derived from rat ventral menencephalon were investigated.

A cell line was established from rat ventral mesencephalon using a retroviral vector containing the temperature-sensitive allele of the SV40T antigen. When cultured with serum-free medium at the permissive temperature, this cell line showed cell death accompanied with DNA laddering in gel electrophoresis and positive in situ staining for DNA 3'-OH end. An addition of bFGF (10 ng/ml) prevented cell death, supported cell proliferation and increased the expression of neoflament in serum-free medium. bFGF (10 ng/ml) and NT3 (10 ng/ml) did not support the survival of the cells in serum-free medium at the permissive temperature.

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454.11 allergic CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) EFFECTS ON NIGROSTRIAL DOPAMINERGIC NEURONS AND BEHAVIOR IN MPTP-TREATED MICE. A. Tomasi,4 E. Lindvist,4 B. Hoffner,4 S. Ogren,5 L. H. Lin,4 and L. Olson.4 1Department of Neuroscience, Karolinska Institute, S-75177 Stockholm, Sweden, and 4Synergon Inc., Boulder, CO 80301.

Allergic glia promotes neuronal growth and survival, but the mechanisms of the trophic effects are still not clear. It has been suggested that trophic substances secreted by astrocytes can modify the sensitivity of neurons to neurotrophins and other trophic factors. In the present study, we have examined whether glial components in MPTP-treated mice, a model of Parkinson's disease, can modify dopaminergic neurons in vitro (Lin et al., Science 260:1130-1132, 1993). We used a novel recombinant GDNF produced in E. coli, which was purified and cloned (Lin et al., Science 260:1130-1132, 1993). We used a novel recombinant GDNF produced in E. coli, which was purified and cloned (Lin et al., Science 260:1130-1132, 1993). We used a novel recombinant GDNF produced in E. coli, which was purified and cloned (Lin et al., Science 260:1130-1132, 1993). We used a novel recombinant GDNF produced in E. coli, which was purified and cloned (Lin et al., Science 260:1130-1132, 1993). We used a novel recombinant GDNF produced in E. coli, which was purified and cloned (Lin et al., Science 260:1130-1132, 1993).

We examined the effects of a single intracerebral injection of recombinant GDNF 24 hours prior to or 7 days after MPTP exposure in C57/BL mice, monitoring neurochemical and behavioral changes. MPTP (50 mg/kg, i.p.) was injected in a volume of 2 ml into the striatum or over the substantia nigra. Animals were monitored for either 5 or 18-20 days postoperatively, and were then sacrificed for HPLC analysis. Behavioral data include significant increases in spontaneous motor activity (freezing, locomotion and motility) in GDNF-treated compared to vehicle-treated animals. HPLC data showed significant increases in dopamine levels in striatum (97%, p<0.001) and substantia nigra (87%, p=0.003) compared to vehicle-treated animals, when GDNF was given in striatum 24 hours prior to MPTP exposure. When GDNF was given into the striatum 7 days after MPTP exposure and sacrifice, increases in dopamine levels were found in striatum (70%, p=0.003) and in substantia nigra (94%, p=0.0001) compared to vehicle-treated mice. Injection over substantia nigra also led to increases in dopamine levels, but only in substantia nigra (64%, p=0.004) compared to vehicle-treated animals. The findings reported here suggest that GDNF may play a crucial role for dopaminergic neurons, reverting effects of MPTP toxicity in this rodent model of Parkinson's disease.

454.12 BEHAVIORAL, NEUROCHEMICAL, AND HISTOLOGICAL CHANGES IN THE NIGROSTRIATAL SYSTEM OF UNILATERALLY 6-OHDA LESIONED RATS FOLLOWING INTRANIGRAL ADMINISTRATION OF GLIAL CELL-LINE-DERIVED NEUROTROPHIC FACTOR. H. T. van der Kolk,5 A. Van der Hagen,5 E. Clark6, and H. J. F. Van den Bergh.5 1Department of Neurosurgery, Karolinska Institute, S-75177 Stockholm, Sweden, and 5Synergen, Inc., Boulder, CO, USA.

Young adult male Fischer 344 rats were unilaterally injected into the medial forebrain bundle with 6-hydroxypoline (10 mg/kg, 4-6 mg/ml), or saline (4 ml). A single injection (7) was used to select animals whose average rotation exceeded 100 degrees/90 min (100% depending to greater than 99% dopamine (DA) depletion in the ipsilateral striatum. Six weeks later, 0.1 to 100 pg of GDNF (Synergen, Inc.) or vehicle was injected intranigral ipsilaterally to the lesion. Amphetamine-induced rotational behavior (Sigma, St. Louis, MO, US) was quantified weekly for up to 5 weeks post-GDNF injection. Rats were sacrificed at 1, 3, and 5 weeks post-GDNF, for tyrosine hydroxylase (TH) immunohistochemistry and HPLC, for the region of the TH immunoneurons. The presence of NE, DA, DOPAC, HVA, 5-HT, and 5-HIAA in the right and left substantia nigra (SN), ventral tegmentum, and striatum. The highest dose of GDNF treated (100 pg) produced a greater than 65% decrease in rotational behavior which persisted for 5 weeks. This dose also produced leave of DA and DOPAC in the ipsilateral SN which were not statistically different from the contralateral, unlesioned side. Vehicle-treated animals showed an marked DA depletion in the ipsilateral SN. In contrast, there was no change in the lesioned side in the ipsilateral striatum. Immunohistochemistry of nigral sections from the 100 pg GDNF dose groups showed vacuoles associated with increased TH-immunoreactivity. These results demonstrate neurochemical and behavioral improvements in unilaterally-lesioned rats following intranigral administration of GDNF, suggesting that GDNF may be a useful therapy for Parkinson's Disease.
454.15
POSTNATAL SPINAL CORD GRABS IN OCULO: EFFECTS OF TREATMENT WITH GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR. K. Troik, B. Hoffner*, D. Russell**, I. Olson, Dept. of Neuroscience, Karolinska Institute, Stockholm, Sweden, and **Synergex Inc., Boulder, CO.

Glial cell line-derived neurotrophic factor (GDNF) is postulated to be a trophic factor for developing dopaminergic neurons. However, the distribution of mRNA for GDNF in the prenatally injured spinal cord is unknown. The intracortical transplantation of neural tissue provides a unique approach to study the actions of trophic factors in vivo. In the case of spinal cord, although prenatal tissue matures well in the anterior chamber, grafts from newborn animals showed limited survival and reduce their size. Grafts from two-week-old animals usually do not survive at all. In our studies spinal cord tissue from newborn (P1) rats was transplanted to the anterior eye chamber of hosts rats after incubation in 200 μg/ml, 10 μg/ml GDNF or vehicle. GDNF in doses of 0.5 μg, 0.1 μg or 0.05 μg was then injected into the anterior eye chamber at days 5, 10, 15 and 20. There was a dose-dependent increase in growth of the transplants induced by GDNF. Indeed, grafts treated with 0.5 μg actually grew to larger than their initial size after grafting. We also transplanted P14 spinal cord to the anterior eye chamber and injected 0.5 μg CD3P at days 5, 10, 15, 20, 30 and 35. Grafts treated with GDNF also responded with increased growth compared with controls, but did not exceed their original size at transplantation. We are currently investigating the immunohistochemical properties of these grafts.

454.17

GDNF was known to enhance the survival and development of midbrain dopaminergic neurons with an EC50 of ~40 ng/ml (Lin et al., Science, 260:1130, 1993). To further define the specificity of the neuroprotective activity of GDNF, we determined the EC50 for promoting the survival of several distinct classes of embryonic chick PNS neurons on immunoprecipitated chick (ESC) neurones, parasympathetic ciliary ganglion (CG) neurons and sensory dorsal root ganglion (DRG) neurons. In addition, we also examined GDNF as a neurotrophic factor for neocortical rat superior cervical ganglion (SCG) neurons. GDNF promoted the survival of CG, CRG and DRG neurons with an EC50 of 9, 13 and 325 ng/ml, respectively. However, these values are several order of magnitude greater than that required for GDNF to function as a dopaminergic neurotrophic factor in midbrain cultures. GDNF at all concentrations tested (100 ng/ml to 10 μg/ml) could not support the survival of rat SCG neurons in serum-free defined culture medium whereas NSG at 50 ng/ml promoted neuronal survival and neurite extension. Hence, although GDNF can promote the survival of some PNS neurons, GDNF appears to be most potent with cultured midbrain dopaminergic neurons.

454.19
MODULATION OF NEUROTROPHIN RECEPTOR TYROSINE KINASE (RTK) PATHWAYS IN THE MESOLIMBIC DOPAMINE SYSTEM BY DRUGS OF ABUSE. D.S. Russel*, M.T. Behrow, K.L. Winder, and E.J. Nestler. Laboratory of Molecular Psychiatry, Dept of Neurology and Psychiatry, Yale Univ Sch of Med, New Haven, CT 06510

The AMP system is an important mediator of neuronal changes due to drugs of abuse in the mesolimbic dopamine system (Nestler, 11,955, 1993). Neurotrophic factors may modulate or mitigate these changes (see accompanying abstract, Behrow, et al.) Various methods are being employed to investigate the role of the neurotrophin RTK pathways, and their regulation by the AMP system, in the changes induced by morphine and cocaine. Chronic morphine treatment in rats increases PLC-infranmuneactivity in the VTA by 20-30% as determined by western blotting. Morphine also appears to regulate ERK1 and 2 differentially, while significant changes in the levels of raf, PI-3-kinase, and pax were not observed. Chronic cocaine, however, decreased PLC by 10-20%. Several proteins that are immunogically like insulin receptor substrate-1 (IRS-1) are detected in rat brain and some appear to be associated with raf receptor complexes. Levels of a 100 kDa IRS-1-like protein decrease in the VTA with morphine treatment, while levels of a 130 kDa form increase in the hippocampus. Studies of regulation of the phosphorylation states and activities of these proteins are ongoing. Several recent studies demonstrate interactions between the AMP system and RTK pathways. This abstract will mediate to make this include our, ERK and CREB. Single injections of CREB antisense-S-cis-2-acetoxybenzoic acid into the nucleus accumbens lead to a 50% decrease in CREB levels, and attenuate Fos-like protein expression and result in rotation behavior in response to an acute amphetamine challenge. This approach should help to elucidate the study of CREB and the various other proteins mentioned above, in mediating the converging actions of neurotrophins and drugs of abuse in specific brain regions.

454.16

In the present study, the effects of single injections of GDNF into the substantia nigra or striatum of five monkeys were investigated using immunohistochemical, neurochemical, and behavioral methods. GDNF was injected using MRI-guided sterile stereotactic surgery into 150 μg of dopamine into the right substantia nigra and 450 μg/18 into the right caudate nucleus. A separate group of animals served as controls receiving equal volume injections of phosphate buffered saline. Monkeys were tested for functional behavioral changes in the post-GDNF infusions. GDNF was seen to produce some behavioral changes in the animals for up to three weeks following the injections. Some decrease in body weight, increase in activity and occasional slurry disturbances were seen in the GDNF injected group as compared to controls. In vivo electrophysiological studies involving local application of potassium using NFAT-coated carbon fiber electrodes showed increased DA release in areas containing caudate nucleus and putamen, ipsilateral to the GDNF injections. HPLC-EC studies of tissue perfusion with dopamine and glutamine of GDNF injected animals showed an increase in DA content bilaterally in animals which had received GDNF as compared to controls. Immunohistochemical studies showed a proliferation of TH-positive neurites in the substantia nigra of the animals receiving intranigral GDNF injections. These findings demonstrate increases in the functional properties of DA neurons upon exposure to GDNF in monkeys and support the hypothesis that GDNF may be a useful therapy in Parkinson's disease.

454.18
INFLUENCE OF NEUROTROPHIC FACTORS ON BIOCHEMICAL CHANGES IN THE MESPOLLIC DOPAMINE SYSTEM ASSOCIATED WITH DRUGS OF ABUSE. MT Berthou*, DS Russell, DW Self, RM Lindsay*, and EJ Nestler, Laboratory of Molecular Psychiatry, Yale Univ Sch of Med, New Haven, CT.

*Hegeneron Pharmacuticals Inc., Tammy, NY

Chronic morphine and cocaine treatments have been shown to regulate levels of tyrosine hydroxylase (TH) and glial fibrillary acidic protein (GFAP) in the ventral tegmental area (VTA) of the adult rat brain. Previous results demonstrated that the morphine-induced changes could be prevented by direct injection of BDNF or NT4 on TH (2.5μg/ml) into the VTA. In contrast, NT1 (5.1ug/day) intuions mimicked these biochemical changes that are characteristic of chronic morphine treatment (Baltiner-Johnson et al, Neurosci Ab 1953). In the present study, all intracerebral paradigms were utilized. The first tested the ability of neurotrophins to reverse the biochemical changes in rats already pretreated with chronic morphine pellets. NT-4 infusion reversed the characteristic increases in TH and GFAP without any effect on these proteins when given alone. NTFT mimicked the morphine induced increase, but was not additive with morphine. The second paradigm tested the effect of concurrent cocaine (15mg/kg twice daily for 10 days) and growth factor treatment. As with morphine, BDNF abolished the cocaine-induced increases in TH levels.

A possible site of convergence for the intracranial actions of neurotrophins and drugs of abuse is the ERK's (extracellular-signal regulated kinases). We are infusing ERK1 and 2 antisense oligonucleotides into the VTA by osmotic micropumps. Initial studies suggest that these interventions can reduce ERK levels detected via immunoblots. These studies, and related studies of other intracranial targets (Russell et al. Neurosci Abs, this volume), will help elucidate the molecular actions that may be common to the neurotrophins and drugs of abuse.

454.20
CONDITIONED MEDIUM DERIVED FROM MESENCEPHALIC TYPE-I ASTROCYTES EXERTS DIFFERENT EFFECTS ON DOPAMINERGIC NEURONS AND PC12 PHEOCHROMOCYTOMA CELLS. J.M. Johnston*, T. Takahashi*, K. Blakely* and J. W. Commission

Previously, we have developed a primary neuronal culture of the ventral mesencephalic region of the E14 rat in which the ventral mesencephalic dopaminergic cells are enzymatically purified and the percentage of dopaminergic neurons is reduced to ~2% at days in vitro (DIV 5). These cultures are thus good models for the biochemical and specific dopaminergic neurotrophic factors. In this study we use our model culture and a PC12 differentiation bioassay to demonstrate specific effects of conditioned medium derived from type-I astrocytes (A-CM) on dopaminergic neuronal survival and function. When cultured in defined medium, 3.8±1x10⁶ neurons/dm² dopaminergic neurons were present at DIV 5. The addition of 10% serum, A-CM or conditioned medium derived from B49 glial cells (B49-CM) increased the survival of dopaminergic neurons present to 42.4±15.9 x10⁶ (p<0.0001) and 36.6±26.7 x10⁶ (p<0.0001) and 8.3±1.9 x10⁶, respectively. Further, B49-CM increases the survival of dopaminergic neurons and promotes the differentiation of PC12 cells, however, the effects of A-CM are specific to the survival of dopaminergic neurons. We are currently investigating the active glycoproteins that we have identified. The true significance of these factors remains to be elucidated.
455.1 A BIOSYSSAY METHOD FOR DOPAMINERGIC NEUROTROPHIC FACTORS BASED ON THE USE OF MICROCULTURES AND IMAGING METHOD: T. Takeda*, G. Ishida, K. Takahashi, J.M. Johnston*, J.W. Commission* Division of Neuroscience, Rush University Medical Center, Chicago, IL 60612. Japan. NTU, 60th, NINDS, NIH, Bldg. 10, Rm S218, Bethesda, MD 20892.

BDNF, GDNF, IL-1β, MGF, POFG-β3, EGF, TGF-α, type-1 α-tropo, type-2 α-tropo, α-2A predominant, microglia, and ischemic insults all promote the survival of dopaminergic neurons in cultures directly or indirectly. Experimental conditions used to test the survival of dopaminergic neurons differ widely among investigators; 1) cell density, 2) fetal age, 3) dissection techniques, 4) type of serum, 5) the age of the cultures when stained and 6) the methods selecting fields for analysis are among the most important. We have developed a method, if used widely, could bring a degree of objectivity to the field, and allow for a comparison of data from different laboratories. In this method, the E14 fetus is used. Only 1.0 mm of ventral mesencephalic tissue, which contains 90% of dopaminergic neurons, is dissected. The growth medium contains 0% of serum throughout. The cells are suspended at a density of 5 x 10⁶/ml. Only 25 μl of the cell suspension is plated per chamber (area covered ~6.25 mm²), as a 90% confluent growth medium. This method is in use for the study of basal ganglia neurotoxicity and the role of NGF, a survival factor for dopaminergic neurons.


The physiologic factors that regulate survival of midbrain dopaminergic neurons have not yet been identified with certainty. Here we show that, transforming growth factors (TGF)-β2 and TGF-β3, at picomolar concentrations, prevent the death of embryonic dopaminergic neurons in culture, and that both factors are expressed in the environment of dopaminergic neurons. The TGF-β2 & 3 could therefore be physiologic trophic factors for midbrain dopaminergic neurons and may be useful as therapeutic agents for Parkinson’s disease.


It is known that while the cholinergic innervation of the rat striatum express the low-affinity NGF receptor (LNGF) during development, the immunoreactivity of these neurons is increased bilaterally following injury to this region. We demonstrate that the LNGF of the adult striatum were increased in response to striatal damage and that this phenomenon is apparently modulated by endogenous NGF. Because immunoreactivity of the LNGF following tissue damage may provide fundamental information on the modulation and function of this receptor, we studied various parameters which might affect immunoreactivity. Injury was administered in one group by implantation of a cannula unilaterally into the striatum (AP=-5.7, L=-2.5, 3.5 mm) and injected and attached to an Alzet 2002 osmotic pump. In addition to injury, this group also received a 14 day continuous infusion of either NGF (300 ng/day) or ACP. Injury was administered in a second group via intra-striatal infusions of acetylcholinesterase (90 or 120 nm) into the striatum. In both cases animals were sacrificed and the tissue was processed for p75 and ChAT immunohistochemistry. Results indicate that contrary to expectations, in neither group was p75 immunoreactivity of striatal cholinergic neurons observed, despite clear immunoreactivity of septal-dorsal band cholinergic neurons in the same sections. Additionally, pronounced hyperactivity of ChAT immuno-reactive striatal cholinergic neurons was seen in the NGF infusion group, demonstrating a biologically effective NGF response in these neurons.

In summary, the lack of striatal p75 immunoreactivity in either the damaged or NGF infected animals, in the face of clear p75 immunoreactivity in the septum, as well as NGF-induced ChAT(+)/acetylcholinesterase, raises more questions than answers. While regulation and function of the LNGF remains an area of interest and intrigue, the new experimental design and biologic activity seen in the present study suggest that an NGF response is more complex than previously suggested.


To date, two key pieces of evidence are lacking to substantiate the hypothesis that NGF may provide an effective means to treat neurodegenerative diseases: (1) better evidence for NGF-enhancement of survival, function or fate of basal forebrain cholinergic neurons under conditions of neurodegeneration (as opposed to atrophy); and (2) a convincing demonstration of direct delivery of NGF to the target cells in vivo. The present studies were intended to address these issues. The neurotrophin AFS64a (referred to as AFS64a) was used to induce degeneration of the basal forebrain cholinergic system. In this first study NGF was continuously infused into the lateral ventricle via an Alzet pump. The results demonstrated a reliable decrease in high affinity choline uptake (HACU) due to AFS64a neurotoxicity and slowly complexing via NGF secretion. These data demonstrate that NGF is able to provide a protective and/or a compensatory response against neurotoxic-induced degeneration of the basal forebrain cholinergic system.

In a second study, we attempted to determine whether similar effects could be achieved through direct delivery of NGF. We studied the effect of a NGF conjugate targeted to the transferrin receptor antibody, OX26. This conjugate has been shown to transport NGF across the BBB. As in the first study, substantial degeneration of basal forebrain neurons was observed in the AFS64a group. In the NGF/OX26 conjugate group was increased by 90% compared to AFS64a vehicle-treated rats. However, immunocytochemistry directed against ChAT and the low affinity NGF (p75) indicated that no increase in p75 immunoreactivity. Thus, we made the following conclusions: 1) the conjugate apparently increased HACU by enhancing the activity (and presumably the viability and function) of surviving neurons while not necessarily protecting against the AFS64a cytotoxicity, per se.

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455.7


The regulation and function of the low-affinity NGF receptor (p75) is an issue of current interest and controversy. To better understand the role of this receptor in basal forebrain cholinergic neurons, we analyzed tissue for changes in immunoreactivity to the p75 in two different rodent models of NGF expression and function: AP54A toxicity and fibroblastng- (FKN) interactions. In the first model, 10 days following the bilateral administration of AP54A to young adult rats, there was a significant reduction in the levels of hippocampal high-affinity choline uptake, as expected. Additionally, the number of septal choline acetyltransferase (ChAT)-immunoreactive neurons was reduced; however, enlarged fibers intensely immunoreactive for p75 were observed in the septum. These fibers were most prevalent in the area dorsal to the anterior commissure, were organized in a dorsoventral orientation and were never associated with an obvious cell body.

Brain sections from animals subjected to FKN lesions were characterized by a loss of acetylcholinesterase activity in the target tissue and both ChAT and p75-immunoreactive neurons in the septum. In contrast to the AP54A lesion, there was no evidence of thick p75 fibers as a result of the FKN lesion. While it remains unclear whether the presence of these p75-positive fibers represents a regenerative response (i.e. sprouting) or evidence of a degenerative response (i.e. tombstone marker), it is likely that an understanding of this phenomenon may provide insight into the regulation and/or function of the p75 low affinity NGF receptor.

455.10

**EXPRESSION OF BIOLOGICALLY ACTIVE NERVE GROWTH FACTOR (NGF) MEDIATED BY RECOMBINANT HERPES SIMPLEX VIRUS TYPE 1 (HSV-1) GENE TRANSFER VECTORS.** K.A. Led*, S.E. Lipton, D.J. Kozak, and J.C. Glorian*. Department of Molecular Genetics & Biochemistry, and Western Psychiatric Institute & Clinic, University of Pittsburgh School of Medicine, Pittsburgh, PA; and Department of Neurology, University of Michigan Medical School, Ann Arbor.

In order to study the effects of neurotrophic factors in chronic neurodegenerative conditions such as Alzheimer's and Parkinson's diseases, we have constructed replication compromised and replication defective (mutant d102) Delucia et al. (1987, J. Viral. 6;558) Herpes simplex virus (HSV-1) gene transfer vectors that express the herpes g B-gene product (v-110) as a supporting factor for human cytomegalovirus immediate early promoter (HCMV-IEP) driving transcription of the murine B-NGF DNA was incorporated into the HSV-1 thymidine kinase (tk) gene region of the recombinant viral vector. The NGF vectors resulted in NGF specific transcription and expression as detected by Northern bio analysis. In vivo expression of the NGF transgene was observed by in situ hybridization two days following stereotactic injection of the expression vector into the hippocampus of rat brains. These results suggest that the NGF vectors are transcriptionally active in vivo. For the length and level of bioactive b-NGF expression in the CNS is in vivo. Thus, we are poised to use these vectors in studies of recovery from fibromyalgia syndromes and excitoisential lesioning of striatum in rat brain as models of chronic neurodegenerative conditions.

455.12

**ASPHYXIA DURING BIRTH MAY PRIME THE CNS. Induction of a long-lasting increase in bFGF gene expression coincidentally with increased mitotic activity in neuronal cell bodies in the substantia nigra.** F. Anderson*, A. Blum, Y. Chen, P. Grenoth, B. Bijlsma, B. Diaz, and M. Herrera-Marschitz. Dept. Internal Medicine, Karolinska Institutet, Huddinge Hospital, 141 86 Huddinge, Sweden; Dept. Neuroscience and Dept. Physiology & Pharmacology, Karolinska Institutet, 6Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, U.S.A.

Asphyxia was induced during birth to male Sprague-Dawley rat pups. A quantitative analysis of the number of tyrosine hydroxylase-immunoreactive (TH-IR) and thrombin-stained nerve cell bodies as well as measurement of basic Fibroblast Growth Factor (bFGF) mRNA levels was carried out in the substantia nigra and the hippocampus on male rats at 3-4 weeks of age. Immunocytochemical analysis of the hippocampus of the control and the bFGF mRNA levels and a concomitant increase in the number of dopamine nerve cell bodies of the substantia nigra. Asphyxia reduced the number of cell bodies in the C/A region of the hippocampus while bFGF mRNA levels remained unchanged.

In conclusion, the asphyxia-induced long-lasting increase in bFGF gene expression may cause the increase in dopamine cell body number in the substantia nigra. The present results indicate that asphyxia during birth can prime the development of the central nervous system in a long-term perspective and may be important in the development of neurodegenerative disorders later in life.
455.13
EVIDENCE FOR NOVEL NERVE GROWTH FACTOR (NGF)-
RESPONDING NEURONAL POPULATIONS IN THE MAMMALIAN
Kodatos**. Departments of Pathology, Neurology, and Neuroscience,
Neuropathology Laboratory, The Johns Hopkins University School of
Medicine, Baltimore MD 21205; Neurogeniatric Research Institute,
University of California, Santa Barbara, CA 93106.
In the CNS, only striatal and basal forebrain cholinergic neurons have been
shown to respond to NGF. Both of these populations are also known
to express the high-affinity NGF receptor, TrkA. This study was
designed to delineate novel NGF-responsive neuronal populations in the
rat brain. Using a hybridization histochemical and RT-PCR, TrkA mRNA
was found in the nucleus prepositus hypoglossi and ventral
torticollis nucleus. TrkA protein was also detected by
immunocytochemistry in the nucleus prepositus and in the dorsal and
ventral torticollis nucleus. In addition, trkA immunoreactivity was seen in the
spinal ventral nucleus, neurons within the medial longitudinal fasciculus, and the
gigantocellular nucleus of the reticular formation. NGF mRNA was present throughout the vestibular and auditory systems with high levels of expression in the cerebellum. These results suggested that NGF might play a role in cerebellar afferent systems. Radiolabeled NGF injected into various sites within the cerebellar cortex was retrogradely transported to the nucleus prepositus and spinal ventral nucleus. The trkA and NGF expression patterns and retrograde transport data together identify novel trophic targets for NGF in the brain and raise the possibility that NGF may be used as a therapeutic agent for diseases of the vestibular and auditory systems.

455.15
COORDINATE EXPRESSION OF NEUROTROPHIN-3 AND TRK C LINKED TO A MORE FAVORABLE PROGNOSIS IN
Children's Hospital, Dept. Cell. and Mol. Biol., Dana-Farber Cancer
Inst., Harvard Medical School, Boston, MA 02115.
We have examined the expression of neurotrophins in medulloblastoma, a malignant brain tumor believed to be derived from the external germinal layer of the cerebellum. Northern and western analyses were performed on tumor samples (n = 11) which were snap frozen in the operating room to preserve RNA and protein integrity. All of the tumors were found to express mRNAs encoding neurotrophin-3 (NT-3) and its cognate receptor, trk C. Due to limited tissue, 3 tumor samples were tested and found to have NT-3 protein and TrkC immunoreactivity. Patients with tumors expressing high levels of trkC mRNA had significantly longer intervals without disease progression than those with low levels (Kaplan-Meier, P = 0.02). Expression of trkA, trk B, or p75 was not associated with favorable disease outcome.

Coordinate expression of ligand and receptor implies the constitutive activation of Trk C receptors in patients with a more favorable outcome. Conceivably, this activation promotes the differentiation of medulloblastoma and may be relevant to the development of new therapeutic strategies for the tumor. At minimum, our findings indicate that trk C expression is a marker of prognostic value.

455.17
NT-3 ATTENUATES REDUCTION IN H-REFLEX PRODUCED BY PYRIDOXINE TOXICITY TO LARGE PRIMARY AFFERENT NEURONS IN
Tarrytown, NY 10591.
High doses of pyridoxine (vitamin B6) are toxic to large primary afferent neurons, producing a peripheral neuropathy in experimental animals that can serve as a model for toxic large-fiber neuropathies in humans. Affected neurons are part of the circuitry that mediates the monosynaptic stretch reflex and a neurophysiologically correlate, the H-reflex. Sprague-
Dawley rats were treated with pyridoxine (400 mg/kg), with or without
additional NT-3 treatment. When half the animals receiving either of these two treatments developed severe deficits on a precise locomotor task, all were tested electromyographically. Animals receiving NT-3 without pyridoxine and non-injected control animals were also tested. We recorded electromyographic activity in the plantar muscles, evoked by stimulation of the tibial nerve. Amplitudes of directly evoked motor (M-)
and reflex-evoked (H+) waves were recorded. M-wave amplitudes did not differ among groups of pyridoxine treated rats (magnitudes normalized to the M-wave amplitude as H/M ratio) were absent or greatly diminished in nearly all animals receiving pyridoxine without NT-3. The reduction was significantly attenuated in anesthetized rats receiving NT-3, resulting in substantial H-reflexes in nearly all animals so treated. H-reflex amplitudes were correlated with performance on the locomotor task. The results are consistent with protection by NT-3 of large sensory fibers from neurotoxic damage, potentially including damage caused by chemotherapeutic agents.

455.18
THE UPERPULATION OF NGF DURING INFLAMMATION PRODUCES PAIN HYPERSENSITIVITY AND AN
INCREASED GROWTH CAPACITY IN PRIMARY SENSORY NEURONS. C.J. Woolf, B. Safihi-Garbadian, T. Leslie, J. Winter.
SPIN: Brain Research Association. Dept. of Anatomy, University
College London, London WC1E 6BT, Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN
The inflammation produced by an intraplantar injection of complete Freund's adjuvant results in a significant elevation in NGF levels, first in the inflamed tissues, then in the innervating nerves. This is accompanied by the development of substantial thermal and mechanical hyperalgesia, and an upregulation in DRG neurons of the mRNA neurotrophine substance P, and CGRP and the growth associated protein GAP-43. Neuronal pretreatment with a neutralizing anti-NGF antibody which does not recognize BDNF or NT3, prevents the development of the abnormal sensitivity and the changes in chemical phenotype, without reducing local edema or erythema. The production of increased levels of NGF in the periphery during inflammation may play a key role in generating inflammatory pain hypersensitivity by increasing transduction sensitivity, amplifying the input to the dorsal horn, sensitizing dorsal horn neurons and promoting a hyperinnervation of the inflamed area by inducing collateral sprouting. Supported by Sandoz and the Sir Jules Thorn Trust.

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455.19 BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) PRODUCES AN ANTI-DEPRESSANT LIKE EFFECT IN TWO ANIMAL MODELS OF DEPRESSION. P. A. Mason, A. Jin, D. N. Green, and S. J. Wiegand. Ronald M. Lindsay. Regeneron Pharmaceuticals, Tarrytown, NY. 10591

We have previously reported that midbrain infusion of the BDNF, a member of the neurotrophin family, produces behavioral and neurochemical evidence of antidepressant-like effects in two animal models of depression, the forced swim test and the learned helplessness paradigm. Adult rats were infused with BDNF (24 ug/day) or PBS vehicle into the midbrain, near the PAG and dorsal raphe, as previously described (Olney et al., 1994). In the learned helplessness paradigm, rats pre-exposed to inescapable shocked showed severe impairments in escape behavior during subsequent conditioned avoidance trials (61% decrease). With the y numbers of escapes, 5 fold increase in escape latency). Midbrain BDNF infusion reversed this escape deficit to levels similar to that obtained from non-shot control rats. No changes in locomotor activity as measured by the total number of 629 partial rotations or grid activity, was seen. These data demonstrate an antidepressant-like action of midbrain-infused BDNF. Furthermore, these results support a role for neurotrophins in the etiology and treatment of neuropsychiatric disorders.

456.1 DEVELOPMENT OF SOMATOSTATIN AND LEU-KNEKPEPHALIN IN THE AUDITORY BRAINSTEM OF THE RAT. M. Koga* and E. Pippin. Univ. Tubingen, Animal Physiology, Auf der Morgenstelle 28, 72076 Tubingen, Germany.

During early ontogeny, the neuropeptides somatostatin and leu-enkephalin are prominent in several brain areas and are therefore thought to be involved in developmental events. This study was focused on the developmentally related expression of these neuropeptides in the auditory brainstem of the rat by means of PAP-immunocytochemistry between the ages of embryonic day 17 and adulthood. During fetal development somatostatin immunoreactive (SOM) neurones appeared in the nuclei of the cochlear nucleus complex (CN), the inferior colliculus (IC), whereas SIR fibres were predominantly found in the superior olivary complex (SOC) and in the IC. In all auditory brainstem nuclei, the immunoreactivity increased developmentally until postnatal day 7. At this age the immunoreactivity in the brainstem was nearly restricted to auditory nuclei, which showed a heavy labeling. The most dense SIR fibre network was found in the SOC, which most probably originated from neurons of the CN, thus providing a system-immanent innervation. Subsequently SIR decreased dramatically until adulthood. In contrast to the transient expression of somatostatin, the number and labeling intensity of leu-enkephalin (LKR) structures increased progressively with age. LKR fibres in the fetal brain appeared in the SOC, NLL, CN and IC. LKR somata were found only after P12 in the CN and in the IC. Our results demonstrate a strong and transient expression of somatostatin and a progressive increase of the leu-enkephalin expression during development. Both neuropeptides are expressed during a period when synaptic stabilization occurs in the rat auditory brainstem, suggesting that they may play a role in synaptic refinement. Supported by the DFG (Pi 727/1-3) and the GKN Tubingen.

456.3 POSTNATAL DEVELOPMENT OF THREE ISOFORMS OF PROTEIN KINASE C IN CENTRAL VESTIBULAR PATHWAYS AND CEREBELLUM OF THE RAT. M. Garcia* and R. E. Haltas. Dept. of Otolaryngology and Anatomy and Neurosurgery Training Program, Tulane University School of Medicine, New Orleans, LA 70112.

Although various protein kinases (PKC) isoforms are highly expressed in brain, little is known about patterns of expression of PKC during development of the CNS. We used immunocytochemistry to study the ontogeny of PKC-β1, β2 and β3 in central auditory pathways of the rat brain, at postnatal (P) days 5, 11, 15, 20, and adult. With the β1 antibody, labeled fibers were found in primary afferents of the VIIIth nerve in all ages.Weakly stained cells in the inferior colliculus nuclei (VCN) at P5 only; no terminals were found in the dorsal cochlear nucleus (DCN), suggesting that the labeled afferents are primarily vestibular. Labeled cells were observed at all ages, with a few labeled cells in the DCN in adults only. Labeled terminals were found in the superior olive (SO) beginning at P15. Cell body labeling was found in the SO and trapezoid body (TB) at P30 and in the lateral lemniscus at P15. In the lateral lemniscus (LL) at all ages, with labeled terminals on neurones of the nucleus of the LL (NLL) beginning at P30. Labeled terminals and a few labeled cells in the DCN were found in the inferior colliculus (IC) at all ages, and in the medial geniculate (MG) at P20 and adult. With the β2 antibody, a few labeled fibers were found in the VIIIth nerve as early as P11, but labeling was strongest at P30. Labeled terminals in the DCN beginning at P11 were present at the same age. Labeled cells were found consistently in the VCN beginning at P15. Cells decorated with labeled terminals were found in the SO beginning at P1. Cytosplastic staining of SO and TB was observed beginning at P11. In the IC beginning at P15. Decorated cells were first observed in the VCN and DCN at P15. Labeled terminals were found in the SO beginning at P11. Labeled terminals were found in the central core of the LA beginning at P15. These results suggest that these isoforms of protein kinase C may play roles in the development and function of central auditory pathways. (Supported by the National Institute of Neurological Disorders and Stroke.)

456.4 POSTNATAL DEVELOPMENT OF THREE ISOFORMS OF PROTEIN KINASE C IN CENTRAL VESTIBULAR PATHWAYS AND CEREBELLUM OF THE RAT. R. E. Haltas* and M.M. Garcia. Dept. of Anatomy and Otolaryngology and Neuroscience Training Program, Tulane Medical School, New Orleans, LA 70112.

The protein kinases C are a family of enzymes which are enriched in brain, yet little is known about their abundance during development of the CNS. We used immunocytochemistry to study the ontogeny of the β1, β2 and β3 isoforms of protein kinase C in the vestibular system and cerebellum of the rat brain, at postnatal (P) days 5, 11, 15, 20, and adult. With the β1 antibody, labeled cells were found in primary afferents of the VIIIth nerve at all ages. Most of these cells were in the cochlear nucleus (VCN) at P5 only; no terminals were found in the dorsal cochlear nucleus (DCN), suggesting that the labeled afferents are primarily vestibular. Labeled cells were observed at all ages, with a few labeled cells in the DCN in adults only. Labeled terminals were found in the superior olive (SO) beginning at P15. Cell body labeling was found in the SO and trapezoid body (TB), beginning at P11. In the lateral lemniscus (LL) at all ages, with labeled terminals on neurones of the nucleus of the LL (NLL) beginning at P30. Labeled terminals and a few labeled cells in the DCN were found in the inferior colliculus (IC) at all ages, and in the medial geniculate (MG) at P20 and adult. With the β2 antibody, a few labeled fibers were found in the VIIIth nerve as early as P11, but labeling was strongest at P30. Labeled terminals in the DCN beginning at P11 were present at the same age. Labeled cells were found consistently in the VCN beginning at P15. Cells decorated with labeled terminals were found in the SO beginning at P1. Cytosplastic staining of SO and TB was observed beginning at P11. In the IC beginning at P15. Decorated cells were first observed in the VCN and DCN at P15. Labeled terminals were found in the SO beginning at P11. Labeled terminals were found in the central core of the LA beginning at P15. These results suggest that these isoforms of protein kinase C may play roles in the development and function of central auditory pathways. (Supported by the National Institute of Neurological Disorders and Stroke.)

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456.2 DEVELOPMENT OF PRIMARY VESTIBULAR AFFERENTS AND VESTIBULOSPINAL PROJECTIONS IN THE OPOSSUM, MONADELPHIS DOMESTICA, J. F. Piétreger* and T. Cabana. Sciences Biologiques, Université de Montréal, C.P. 6128, Succ. centreville, Montréal, Canada, H3C 3J7.

The opossum Monodelphis domestica is born after only 17 gestational days. It is then very immature (eyes and ears closed, budlike hindlimbs, etc.) but has the capacity to locomote on the mother’s belly with its forelimbs, hand the genital aperture to a nipple for sucking until postnatal day 28. This behavior of the newborn suggests that some sense systems must be present to guide it. One such system may be the vestibular system, which is known to mediate sensory clues about head movement and which has a strong influence on posture and balance during locomotion in adult mammals. We have looked at the development of the primary afferents to the vestibular nuclei and the vestibulospinal projections to the spinal cord using the neuroanatomical tracing of BI and LET-DH. We found that VIIIth nerve fibers have attained the vestibular nuclear complex at birth and that some of the fibers project to the contractral vestibular ganglion, a projection which was not found in the adult opossum. Projections from the lateral vestibular nucleus, and, to a much lesser extent, parts of the median and the inferior vestibular nucleus, to the brachial and lumbar sacral enlargements of the spinal cord are also present on the day of birth. Without excluding the possibility of olfactory and/or tactile guidance for directing the “climbing” behavior of the newborn opossum, the vestibular system is likely involved.

The trapezoid body decussation carries information that is essential to the localization of sound by the mammalian auditory system. The trapezoid body decussation consists of fibers from the ventral cochlear nucleus that terminate in the contralateral superior olivary complex. The purpose of this study was to establish the developmental decussation and to examine the relationship of the fibers and their growth cones to potential cues during pathfinding. The carboxyamine dye DL was placed in the area of the presumptive olivary nuclei of P8-12 mice, in order to label the different fibers and their growth cones. At E13, although cochlear nucleus neurons were just beginning to migrate away from the rhombic lip ventricular zone, they were already sending axons along the marginal edge of the hindbrain towards the midline. By E15, many fibers had entered the midline region and some appeared to contact glial cells at the midline. In addition, by this time some of the fibers had crossed the midline and had reached the vicinity of the presumptive superior olivary complex on the contralateral side. At E17, the presumptive cochlear nucleus could be clearly distinguished on the lateral surface of the hindbrain and there was a noticeable increase in the number of fibers in the trapezoid body decussation. Based on growth cone morphology and axon anatomy, it does not appear that all trapezoid body fibers depend on the basal lamina for guidance during pathfinding. In addition, growth cone complexity did not show any consistent increase in the floorplate region. Growth cone contact of glial cells at the midline is an important role in directing fibers across the midline to their targets. Supported by NIH DC003155.

5.6.6 A TRANSIENT DEVELOPMENTAL GRADIENT OF GLYCINERGIC ENDINGS IN THE FERRET SUPERIOR OLIVARY NUCLEI. C. K. Hitt, J. K. Brunso-Bechtold, and C. K. Hitt, Dept. of Neurobiology and Anatomy, Neuroscience Program, Wake Forest University, Bowman Gray School of Medicine, Winston-Salem, North Carolina 27157.

The development of inhibitory connections from the medial nucleus of the trapezoid body (MNTB) to the lateral (LSO) and medial (MSO) superior olivary nuclei was investigated in ferrets using immunocytochemistry with antibody for glycine (courtesy of D. R. Westphal). We were particularly interested in whether or not the glycergic gradient was established by a tonotopic gradient. Videograms of LSO and MSO in selected innervated sections at 7 postnatal ages were digitized. Samples of the neuropil were analyzed and averaged to determine the relative density of glycine immunostaining. Only a diffuse background staining is immunohistochemically observed in the neuropil at postnatal day 7, but by the end of the second postnatal week immunostaining in the neuropil is more granular. Between the third and fourth postnatal weeks, the neuropil has the characteristic puncta of immunostained endings in the adult. A gradient is present across the tonotopic axis of both nuclei during the earliest period of diffuse immunostaining. At this time, there is a near two-fold increase in density of immunostaining from low to high frequency regions. By postnatal day 28 when puncta are evident, glycine immunostaining is homogeneous all along the tonotopic axis. Glycine-immunopositive cells in the MNTB appear progressively along a spatial gradient during the first postnatal month as this maturation of glycnergic terminals in LSO and MSO is taking place.

Supported in part by NIH grant DC003155.

5.6.7 FREQUENCY-DEPENDENT ADJUSTMENT OF BINURAL TUNING PROPERTIES IN THE FERRET ON OWLS OPTIC TERRITORY: A RESULT OF ALTERED AUDITORY EXPERIENCE. J. T. Gold, and E. J. Knudsen, Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

Barn owls localize sounds by combining auditory cues, including interaural differences in timing (ITD) and level (ILD), which are computed across a broad range of frequencies. The auditory space map in the optic tectum (OT) contains neurons sensitive to external sound stimuli from external sources and from external receptive fields. Previous studies have shown that, in the OT, neuronal tuning to ITD and ILD for narrowly frequency-limited sound stimuli in the region corresponding to the unit's receptive field. We examined the extent to which each frequency-dependent tuning is shaped by experience by testing the ability of neurons in the OT to respond to ITD and ILD levels under novel, frequency-dependent localization cues. An ear plug-like device ("twisted tube") was developed and shown via auditory microprobes microinjected in the optic tectum to affect the timing and level of sounds in a frequency-dependent manner. Juvenile owls were trained on 60 dB with a twisted tube in one ear. The results showed that neuronal tuning to ITD and ILD levels was significantly changed. First, neurons that were tonotopically-predicted, broadband stimuli were characterized at a variety of sites. The frequency-dependence of both ITD and ILD tuning was shown to reflect the frequency-dependence of the sound stimuli caused by the twisted tube. Second, with the twisted tube removed, the auditory receptive fields measured with broadband stimuli of different frequencies were shown to be misaligned in both azimuth and elevation, corresponding to the frequency-dependent changes in ITD and ILD tuning, respectively. Re-insertion of the device realigned the receptive fields, thereby demonstrating the compensatory nature of the adaptive adjustments. Thus, experience-dependent plasticity shapes the frequency-dependence of unit tuning to ITD and ILD of neurons in the barn owl's optic tectum. Supported by NIH: 3752 MH11074 and R01 DC001515-14.

5.6.8 TOPOGRAPHY OF THE PROJECTION FROM THE CENTRAL TO THE EXTERNAL NUCLUSES OF THE INFERN COCCULUS IN NORMAL AND PREMATURE-BORN BARN OWLS. E. H. Klionsky, Dept. of Neurobiology, Stanford University, Stanford, CA 94305-5401.

The central nucleus of the inferior colliculus (ICc) contains neurons sensitive to interaural timing difference (ITD), the primary cue used by the barn owl to localize sounds in azimuth. ICc neurons are organized into a map of ITD where the most central region of ICc is most sensitive to ITD and the peripheral regions are more sensitive to ILD. ITD neurons project to ICc via a topographic projection from ICc to ICc. Rearing owls with laterally displacing prismatic spectacles in adult life did not show the same pattern in ICc as in the normal owls, but not in the ICc, suggesting that the projection from ICc to ICc is altered by prism-rearing. This hypothesis was tested by making small injections of biotinylated dextran amine (BDA) at a variety of locations in ICc and frontal and prismatic-reared owls. R departed labeled ICc neurons were visualized by avidin-biotin-peroxidase histochemistry. The positions made in ICc were measured along the rostrocaudal axis of the ICc (the axis of varying ITD), relative to the calbindin immunostaining of the ICc complex.

Injections (n=10) were made at the level of 0 μsec ITD in the ICc of normal normal labeled ICc neurons (n=149) in the rostral one-third of the lateral shell of the ICc, consistent with the known projection from the ICc to the ICc. Injections (n=13) made in the ICc of prism-reared owls at the same anatomical location, which in these birds was tuned to 40-50 μsec contra-ear leading ITD, labeled ICc neurons (n=245) in a significantly different distribution. About half of the labeled neurons were situated caudally in the lateral shell at locations representing ITDs corresponding to the prismatically induced shift in tonal auditory map. The remaining neurons were situated in the rostral third of the lateral shell, as in normal birds. These preliminary data suggest that the adaptive alteration in ITD tuning observed in the ICc of prism-reared owls results at least in part in an alternation in the projection from ICc to ICc.

Supported by NIH ROI DC-001515-14. D.E.P. is a Howard Hughes Medical Institute Predoctoral Fellow.


Retinal axons can be induced to innervate the auditory thalami followin neonatal lesions in ferrets. We have now shown that induction of retinal projections into the medial geniculate nucleus (MGN) critically depends on extensive afibrillar axonal arborizations extending from both dorsal and ventral retina, as well as from other auditory brainstem nuclei.

To examine the pattern of retinal innervation of the MGN, adult "treated" ferrets received binocular injections of cholera toxin B subunit (CTB). The procedure stains axons and terminals in detail, thus allowing reconstruction of single axons. Retinal fibers can invade all the subnuclei of the MGN, but consistently avoid its caudal part. The ventral and the medial divisions are the most densely innervated. In general, axons innervating the ventral division enter the MGN directly from the optic tract. These axons have an average 6 - 8 μm diameter, are more highly branched, and have large clumperted boutons. Retinal axons innervating the medial division enter the MGN through the lateral posterior nucleus (LP), the precocious nuclei (PF) and the lateral terminal nuclei. They are the most prominent in the MGN, with boutons and sparsely terminal arborizations. Arteries entering the MGN through the LP and PF can also arborize in the dorsal division and in the posterior nucleus (Po).

By injecting WGA-HRP into one eye and CTB into the other, we find that both eye projection to the same areas of MGN and the LP, the ipsilateral projection being less dense. The patterns of projections formed by one eye are adjacent to each other, but closely segregated, from those formed by the other eye. In LP, the projection from each eye appears band-like. These data suggest that while the site of retinal innervation into the MGN, these results reflect the existence of functional and anatomic zone differences. Supported by EY07319, the March of Dimes and MEC EXRT 2550728.

5.6.10 CHANGES OF AUDITORY EVOKED POTENTIALS IN RESPONSE TO BEHAVIORALLY MEANINGFUL TONES INDUCED BY ACUTE ETHANOL INTAKE IN ALTRICIAL NESTLINGS AT THE STAGE OF FORMATION OF NATURAL BEHAVIOR: L. I. Alexandrov, Y. T. Alexandrov Inst. of Higher Nervous Activity & Neurophysiology and Inst. of Psychology, Russian Acad. of Sci. Moscow, Russia.

Acute ethanol's influence on f i l d auditory evoked potentials (AEP) was studied in 6-7-day-old altricial nestlings of the field flycatcher. Nestlings were presented with bi-temporal, non-tonal stimuli, at a frequency index (MI) of the AEP in response to "behavioral" but not to control frequencies. This effect was first observed on day 0, when the nestlings' behavior became more complex: their eyes opened and defense behavior appeared, and when previ-ously formed feeding behavior was undergoing modifications. The MI increase during the early postembryonic ontogeny was probably due to the selective involvement of neurons with newly formed behavioral specializations into the subserving of new behavioral patterns while the decrease of MI under alcohol was due to the depression of activity in these neurons.
SYNAPTOSPHERE EXPRESSION IN THE DEVELOPING INNER EAR OF THE CHICK. A.M. Cunningham* and B.A. Kondoh. Garvan Institute of Medical Research, Sydney, NSW 2010, Australia and University of South Florida, Tampa, FL 33612.

Development of the auditory and vestibular systems is closely associated with early morphological and physiological changes in the inner ear. Studies of synaptosome expression have been used to identify specific neurotransmitter release sites. In this study, we investigated the expression of synaptosomal proteins in the developing chick inner ear to determine their role in the development of the auditory and vestibular systems. We found that the expression of synaptosomal proteins in the inner ear is dependent on the stage of development and that the proteins are required for the normal development of the auditory and vestibular systems.

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DEVELOPMENT OF THE AUDITORY AND VESTIBULAR SYSTEMS

Wednesday PM

456.11

SYNAPTOSPHERE EXPRESSION IN THE DEVELOPING INNER EAR OF THE CHICK. A.M. Cunningham* and B.A. Kondoh. Garvan Institute of Medical Research, Sydney, NSW 2010, Australia and University of South Florida, Tampa, FL 33612.

Syntapsphere is the major integral membrane protein of presynaptic vesicles and is useful as a marker for synaptic terminals. The glycoprotein may be involved in presynaptic functions such as vesicle fusion. In this study, syntapsphere has been implicated as having a specific role in neurotransmitter release (Adler et al., 1992). The expression of syntapsphere was examined during development using monoclonal antibodies (mAb 20.11) which recognize the avian homologue of syntapsphere (Cunningham & Jeffrey, 1990, Soc. Neurosci. Abstr. 16, 1296).

We examined the expression of syntapsphere in the chick embryonic day 6 (ED 6) to posthatch using an immunoperoxidase technique. In the cochlea, syntapsphere immunoreactivity was demonstrated as an ED 8 in the proximal region of the sensory epithelium. In the organ of Corti, immunoreactivity was observed on the regions containing the tectorial and intermediate hair cells. As development progressed, ED 10, immunoreactivity also appeared in the distal regions of the cochlea, consistent with later maturation of this region. In the vestibular endorgans, the saccule, utricle and cristae ampullaris, immunoreactivity was observed at ED 14, the earliest age studied.

Previous studies in mammals have suggested that syntapsphere is expressed primarily in the nerve fibres innervating the cochlea as well as the afferents of the peripheral vestibular system (Gil-Lozoya & Pujo, 1988; Scarfone et al., 1991; Nadol et al., 1993). In the present studies, we demonstrate a pattern of syntapsphere immunoreactivity in areas which primarily receiveafferent innervation and would be consistent with localization to the base of the hair cells. Immunofluorescence studies are needed to verify these findings and to determine the relationship between the expression of syntapsphere immunoreactivity and the events of synaptogenesis and maturation. Supported by grants from The Medical Foundation, Univ. of Syd. to AMC & NIH/NIDCD NS 529 DC01923-01 to BMB.

456.13


Visual signals appear to play an instructive role in the development of the map of auditory space. In the superior colliculus (SC), identified by the site at which this visual auditory interaction occurs has yet to be determined. We have examined the contribution to the development of the auditory space map of the visual representation in the corresponding superficial layers of the SC in the ferret.

The causal region of the superficial layers of the right SC was removed by aspiration at PND 17. Recordings were made bilaterally when the animals were fully grown. On the left, unoperated side, both the visual representation in the superficial layers and the auditory representation in the deeper layers appeared to be normal. On the right side, the visual responses recorded from the superficial layers in normal SC had normal receptive field locations that covered a restricted region of anterior visual space. Auditory units recorded in the deeper layers of the same electrode were much smaller and had corresponding locations, suggesting that the auditory representation in this part of the SC was unaltered. However, the preferred sound directions of auditory units recorded in more caudal regions of the SC, where the superficial layers were missing, were much more scattered, even though many of these units were also visually responsive.

These data suggest that visual responses in the superficial layers of the SC may contribute to the development of the auditory topography in the deeper layers.

DEVELOPMENT OF VISUAL CORTEX III

457.1


We are interested in factors that control the formation of visual corticocortical connections from area 17 to area 18 and the reciprocal projections to area 17. We examined the role of layer 1 in these processes. From the earliest postnatal ages, apical dendrites of pyramidal cortical cells project to layer 1, where they connect with long horizontal fibers that cross several cortical areas.

We lesioned layer 1 in area 18 in newborn kittens, let them develop to 1 month of age and examined cortical projections after labelling them with horseradish peroxidase (HRP) and Fast Blue. In normal kittens, by the end of the first postnatal month, fibers projecting from area 17 to area 18 terminate in patches in the cortex that project back from area 18 and cells projecting back from area 18 are grouped in clusters, mainly in superficial layers with a few in deep layers. In lesioned animals, very few projections into areas 17 and 18 in Nissl-stained sections or cytochrome oxidase-stained cortical sections were observed.

Thus, the lesions produced a disproportionate loss of corticocortical connections that is not easily explained on the basis of generalised cortical damage. Furthermore, the results indicate that intercellular signaling via layer 1 is important for the development of corticocortical connections in the visual cortex.

457.2

DEVELOPMENT OF LONG-RANGE HORIZONTAL CONNECTIONS IN FERRET PRIMARY VISUAL CORTEX. Edward S. Ruthazer* and Michael P. Strzyz. W.M. Keck Center for Integrative Neuroscience and Neurorehabilitation Program, UCSF, San Francisco, CA 94143-2444.

The specificity of long-range tangential connections emerges during development from initially diffuse connections. In the cat, manipulation of visual experience influences only the late portion of this refinement. To examine the activitydependence of the early component we have studied the ferret.

Focal injections of biocytin into the subiculum of collateral (CTB) or CTB-gold conjugate were made into area 17 of ferrets ranging in age from PND 20 to adulthood. Following two to four days post-injection survival, animals were perfused and their occipital cortex flattened for sectioning parallel to the pial surface. In all but the youngest ferrets studied (PND 14 and older), small injections of CTB into superficial cortex resulted in clusters of retrogradely labeled neurons, the axons of which can be traced back to the injection site, and also in many cases to nearby sites of dense axonal arbors labeled in the same injection site. In the adult ferrets, the clusters of labeled neurons appeared coincidently, but not always, with clusters of labeled cells. All at ages studied, several clusters of labeled cells were also found in areas 19, 10, and suprasylvian cortex.

In adult animals, the clusters in area 17 were most prominent in layer III where they formed discrete groups of cells, generally within 3 mm of the injection site, separated by regions of relatively sparse labeling. In the young ferret, the clusters were larger, and more frequently included both the injected sites, and were spread in a more scattered pattern. In the adult ferrets, the clusters of labeled neurons were observed following injection on PND 29. The degree of clustering of labeled neurons was similar to that observed in the young ferrets, with the largest clusters observed in the case of injections on PND 29.

Activity blocked by intracortical TTX injection from PND 23 until sacrifice two weeks later appeared to disrupt normal cluster formation in area 17. Supported by NSF Training Grant EY01710 and NIH Grant EY09760.

456.12


In one set of experiments, we examined the development of projection from the superior olive (MSO) to the inferior colliculus (IC) by examining Fluoro-Gold into the IC unilaterally at postnatal days 0 (P0), 3 (P3), 7 (P7) and adult. They were killed 1 day after injection. Retrogradely labeled neurons in the MSO appeared on the ipsilateral side only in any cases. The labeled frequency of MSO neurons was increased stepwise (from 35% to 50%) with postnatal steps, suggesting differential growth of early- and late-developing axons.

In another set of experiments, we found that the MSO was performed in rats, of which IC had been unilaterally ablated between P0 and maturity. Upon reaching adulthood, rats received injections of Fluoro-Ruby into the contralateral IC and that the labeled axons crossed the midline to the intact IC could be examined. These rats were euthanized 2 days after injection.

1) No exuberant projections to the contralateral IC are found in the normal development. 2) When the IC is ablated unilaterally, many neurons die in the ipsilateral MSO as a result of axotomy. 3) An abberant crossed projection occurs in a few of the survived MSO neurons only in the P3 ablation cases. 4) Growth of late-developing axons is a major factor of plasticity in this system.
DEVELOPMENT OF CLUSTERED LATERAL CONNECTIVITY IN MACAQUE VISUAL CORTEX. K. Takeishi.1, K. Tsumoto.1, J. K. Durack.2,3, S. J. Durack.2,3, C. A. J. Durack.2,3. 1Koganei Mind/Brain Institute, Johns Hopkins University, Baltimore, MD 21211. 2The Brain Research Institute, University of California, Los Angeles. 3Department of Neurology, University of California, Los Angeles.

The clustered lateral connection network in the visual cortex is thought to be a substrate for the parallel information processing underlying visual perception, and is mainly present in layers 2-3 and 5. To examine this hook circuitry, and to identify whether the visual experience influences cluster formation, the anatomical organization of striate cortex (area V1) in the infant and adult macaque was investigated using DI as a tracer. Following the placement of DI crystals at various depths of gray matter, lateral spread of axons and densely branched dendrites of pyramidal neurons was observed in both superficial and in infragranular layers at 140 days, approximately 3 weeks before birth. In the supragranular layer, labeled axonal terminals and somata formed clusters, whereas, in the infragranular layer, a continuous lateral spread of labeled axons was visible without noticeable clusters. These data suggest that the patches seen in layer 5 of adult V1 develop by elimination of axonal branches in later stage. Cytochrome oxidase (CO) histochemistry revealed no evidence of stained dots despite the presence of clustered terminal patches in supragranular layers. From these observations, it was concluded that the visual experience is not necessary to form clustered horizontal connections in macaque V1, and that CO does not guide axons to the proper termination site of intrinsic neurons, but rather blocks are expressed after the time of the formation of patchy connection system. (Supported by NIH grant EY06432).

5.6


We have previously shown that, following neonatal monocular enucleation (ME), the adult rabbit visual (V1) CC distribution is exuberant, extending into areas of cortical regions which do not normally contain callosal cells, similar to the CC distribution in the neonate. We have also shown that levels of noradrenaline (NA) increase in the normal development of the CC distribution. There is a decreased tangential extent of the CC distribution in the adult rabbit visual cortex following administration of an alpha-2 receptor antagonist, yohimbine, during the critical period of ontogeny. We presently examine the effects of yohimbine administration on the CC distribution following neonatal ME. Rabbits (N=4) were deeply anesthetized and had ME on the day of birth. Each animal received injections of yohimbine HCl (2.5 mg/kg, IP) every day from postnatal day 5 through 12. A control group (N=3) had ME and received equal volumes of IP injections of saline. Rabbits were reared until adult, at which time multiple injections of HRP (Boehringer, 20% in H2O) were made (total volume injected: 7 μl) throughout one entire visual cortex. Animals were perfused 24 hours later and the brains were cut and reacted with TMB. ME plus saline animals had a significantly increased tangential extent of the CC distribution, similar to previous reports. ME plus yohimbine animals had a restricted tangential extent of the CC distribution in area 17 similar to the normal adult pattern. Yohimbine administration during the critical period prevents ME induced CC exuberance. Results indicate that NA influences developmental plasticity of visual callosal cells. Supported by NIH NS25689 and DA06681.
CHANGES IN CYTOCHROME OXIDASE-RICH PATCHES IN STRIATE CORTEX OF HUMANS WITH RETINAL LESIONS.


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We studied the tangential distribution of cytochrome oxidase (Cyto)rich patches in striate cortex of two humans with retinal lesions. Patch spatial density and patch cross-sectional area were analyzed in Cyto-rich tangential sections of flat-mounted preparations of V1. Rows of patches are more conspicuous in the cortical representation of the retinal lesions than in normal areas. The spatial density of patches remains constant in the binocular field representation in V1, although patch size changes drastically in the region of representation of the retinal lesion. In this region, patches are larger and darker above and below the ocular dominance stripes of the normal eye than in the alternate stripes. After long-term lesions, the patches corresponding to the normal eye columns appeared larger than in normal controls. In contrast, patches corresponding to the columns in the retinal lesion representation appeared smaller than in normal controls. These results suggest the existence of competitive interactions which modify the cortical intrinsic organization in adult humans.

Financial support: CNPq, FINEP, CAPES, CEPG/UFRJ.

TRANSPLANT- AND PROSTHESIS-ASSISTED REGENERATION

458.1 ELECTROPHYSIOLOGICAL RECOVERY AFTER SPINAL CORD INJURY USING CARBOXYL GROUP ENRICHED FILAMENT IMPLANTS AND TENDON 2746 S SUPPORT.

T. Kamada, M. Dauvard, A. Liu, and C. Tausch.

Rehabilitation R&D Center, Hines VA Hospital, Hines, IL 60141.

Our previous studies have shown that carbon fibers are capable of supporting neurite growth by providing a favorable attachment surface and directionality to regrowing axons. Axons have been found to exert a trophic influence on both the central and peripheral nervous systems. The purpose of this study was to determine whether the implantation of carbon fibers and/or the administration of CMAP (a sub-stimulated ACTH 4-9 analog) would have any beneficial effect after spinal cord injury.

Rats were anesthetized and a total transection of the spinal cord was performed at the T8-T9 level. For animals receiving carbon filament implants, a bundle of approximately 10,000 carbon fibers of 5 μm diameter (AMOCO Themed™) were placed into the transection site. For animals receiving CMAP (50 μg/kg) or CMAP and ACTH (10 μg/kg), the solution was injected into the central canal of the spinal cord at the level of the transection site. All of the five spinal cord transected animals, which received carbon filament implants and CMAP and ACTH, showed significant behavioral recovery (grade 5) at 5 months post-injury. All of the five spinal cord transected animals without CMAP and ACTH showed significant behavioral recovery (grade 5) at 3 months post-injury.

All the reported results were statistically significant (P<0.05) compared to the control group. These findings are consistent with our previous studies on the role of a trophic factor, NGF, in the regeneration of nerve fibers. This study supports the hypothesis that carbon fibers and/or CMAP and ACTH are capable of promoting the regeneration of nerve fibers following spinal cord injury.

458.2 CARBON FILAMENT IMPLANTS ENHANCED WITH GROWTH FACTORS SECRETING FIBROBLASTS PROMOTE REGENERATION OF INJURED SPINAL CORD FIBERS.


Rehabilitation R&D Center, VA Hines Hospital, Hines, IL 60141.

Neurotrophins are proteins which are essential for the survival and maintenance of a variety of different populations of neurons. Among the neurotrophins, nerve growth factor (NGF) is the best characterized. NGF infusion into certain types of neural tissue results in a dramatic outgrowth of nerve fibers. Recent studies have shown that NGF has an effect on a broader spectrum of neuronal populations than was originally believed. Carbon fibers have been shown to provide a substrate for the outgrowth of injured spinal axons. The goal of this study was to determine the effect of NGF-secreting fibroblasts on the regeneration of injured spinal cord fibers.

Fibroblasts which were genetically modified to secrete NGF were cultured on carbon fibers attached to the bottom of petri dishes. After 24 hrs, the fibroblast-coated carbon fibers were implanted into the lesion site of spinal cord contused Fischer 344 rats. All animals were cared for according to IACUC guidelines. Animals were killed 4 weeks post-injury. The results of this study indicate that the implantation of NGF-secreting fibroblasts, grown on carbon fibers and implanted into the contused spinal cord, would promote transition growth of injured spinal cord fibers.

Supported by funds from the Veterans Administration, Rehabilitation R&D Service and the AMOCO Foundation.

458.4 DISSOCIATED, CULTURED SCHWANN CELLS SUPPORT AXONAL GROWTH WHEN IMPLANTED INTO THE SPINAL CORD OF ADULT RATS.


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The ability of cultured Schwann cells to support axonal growth in the spinal cord has been investigated using adult rats. Schwann cells were cultured from the sciatic nerves of newborn rat pups. They were then harvested and injected into small tubes (1-mm diameter; 5-7 mm long) made of polycarbonate film. After a laminectomy, a lesion was made in the dorsal half of the thoracic spinal cord (total length 5-7 mm) and a tube was implanted. In other animals empty tubes were implanted as controls. 1-5 weeks after the animals were perfused and longitudinal sections were cut from the tube and the adjacent spinal cord. Sections were then immunolabeled with antibodies specific for axons, astrocytes and basement membranes. After implantation of 11 days or longer the tubes are filled with cells, many of which have a spindle shape typical of cultured Schwann cells. Neovascularization is evident. Axons can be seen streaming into both ends of the tubes. With short survival times (<2 weeks) these axons extend 2-3 mm toward the center. With longer survivals axons are seen throughout the full length of the tubes. Many are grouped in fascicles while others appear as single fibers. Astrocyte labeling reveals a sharp border between the host tissue and the ends of the tubes. These results provide additional evidence that cultured Schwann cells can support axonal growth in the adult central nervous system.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994

Experiments in female Long-Evans rats tested the suitability of a mixed tissue of CNS and PNS components to support axon elongation. A transected optic nerve was cut and inserted into the end of a silicone tube and a peripheral nerve graft (PNG) was tutured in the opposite end forming a 3-mm chamber gap. The chambers were resected after various time periods post-implantation and the contents of the chambers were studied in serial cross sections to assay the growth across the gap. Early events in these chambers are similar to the analogous PNS chambers with the chamber within one day macrophages, erythrocytes and other vascular components exude from the stumps. A fibrin matrix forms a suspended, coaxial spindle-shaped bridge across the gap. During the second week, although growing axons grew retrogradely through the PNG but advanced further than the end of the graft inserted into the tube, suggesting that mixed PNS and CNS tissue does not support axon regeneration. Supported by the Canadian Spinal Research Organization.

AXONAL OUTGROWTH FROM AN INTRASPINAL PERIPHERAL NERVE (PN) GRAFT PROMOTED BY SUBSTRATE-BOUND NEUROTROPHIC FACTORS (NTFs) [J.H. Ye and J. Houle*]. Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205

One month following spinal cord injury, PN grafts to repair injured central nervous system (CNS) pathways has been the reluctance of regenerating axons to extend beyond the distal end of the graft. Substrate bound NTFs (BDNF, NT-3, NT-4, basic FGF, Regeneration Promoting Factor, RPF) were used to clarify whether the CNS environment adjacent to a PN graft insertion site could be made more favorable for the growth of axons. Following hiruection of a mid-cervical spinal cord of adult rats, a segment of autologous PN was apposed to the rostral cavity surface. The distal end was ligated and left unapposed for 2 weeks, then apposed to a strip of NTF-treated nitrocellulose membrane implanted into the spinal cord 5 mm caudal to the lesion. Untreated nitrocellulose served as a control. Five weeks later the PN graft was cut at midpoint and the caudal end exposed to HRP and the rostral end exposed to True Blue to label neurons contributing to the graft. NTF-treated bundles of labeled axons that had grown down to the ventral edge of the implant, with some fibers extending into the adjacent immediate gray matter. Outgrowth associated with BDNF or NT-3-treated implants was less robust in terms of length and number of regrowing axons than with CNFT, yet was greater than with untreated nitrocellulose implants where very few axons extended beyond the PN graft. True Blue-labeled neurons were prominent in the spinal cord, raphe area, reticular formation and vestibular nuclei, the distribution of which was the same with the different NTFs used. These results indicate that regenerating axons of supraspinal neurons can be induced to extend beyond a PN graft, back into the spinal cord, by a variety of substrate bound NTFs, thereby increasing the potential for establishing contact with host neurons. Supported by NIH grant NS 26380.

HUMAN SCHWANN CELLS CAN ENHANCE AXONAL REGENERATION AND MYELINATION IN THE NUDE RAT SPINAL CORD. J.D. Guest & R.P. Bunge. The Miami Project and Dept. of Neurological Surgery, Univ. of Miami School of Medicine, Miami, FL 33136

Human Schwann cells (HSC) can be purified from donor peripheral nerves; such HSC have been shown to enhance regeneration and to provide myelination for regenerating axons when seeded into semipermeable guidance channels in combination with Matrigel and then placed into gaps spanning sciatic nerve lesions in immunocompromised rodents (Levi et al., J. Neurosci. 14:1309-1319, 1994). Furthermore it is known that syngeneic Schwann cells transplanted within semipermeable guidance channels spanning a thoracic spinal cord lesion promote axonal regeneration from locally implanted axons with cells bodies up to 9 segments rostral and provide myelination for many regenerating axons (X.M Xu et al. Soc.Neurosci.Annual Meet. 1995). We have now evaluated whether human Schwann cells can have similar effects on the regeneration and myelination of CNS axons. In this series of experiments we have placed closed ended (caudal) guidance channels filled with HSC and capped with Matrigel (30:70 vol/vol) contact with the T8 spinal segment of adult female Nude rats. We provide evidence that the HSC survive in contact with the T8 nude rat spinal cord and promote regeneration of axons from various neuronal populations (including brainstem DBH positive neurons) into the channel and provide myelination to large numbers of regenerating axons within the closed ends 4 days after surgery. Because numbers of HSC obtained from human donors are limited methods to expand HSCs to large numbers at high purity have been developed (Levi et al. see abstract this meeting). After 3 passages with mitogens the HSC used within guidance channels appear as competent to recruit regenerating fibres and provide myelination as non-expanded HSC. Dr. Guest is a Fellow of the


SCs support regeneration of axons from spinal cord neurons into guidance channels grafted into transected adult rat spinal cord (Xu et al, '92, '93). We investigated if axonal regeneration is improved in SCI and in SCI treated with methylprednisolone (MP), is administered when SC grafts are implanted. SCI were perfused in culture from adult rat spinal cords, survived in Matrigel/DMEM (30:70), and were seeded into semipermeable PAC/PVC channels at a density of 120 X 10^6 cell/ml. MP (30 mg/kg) or vehicle (control) was injected i.v. at 5m, 2h, and 4h after transection at T8 and removal of 1-3 caudal cord segments. Either the rostral stump was inserted 1.5mm into a channel capped at the caudal end, or both stumps were inserted into an open-ended channel. One mo later, the MP + SCI-chopped channel group, compared with controls, had larger tissue cables, improved blending of host cord and graft tissue, more myelinated axons (n=6; x=1159 vs. 500) in the graft, and labelling of more spinal cord neurons (n=1128 vs. 280) after injection of Fast Blue into the graft (n=4). Also, in contrast to the control, 5-HT+ and DBH+ axons were detected in the graft, and brainstem neurons (n=34) extended axons into the graft as determined by Fast Blue tracing. When the channel was open at both ends, 45-60 d later the mean of myelinated axons in the graft was 3237 (n=5; control, 1217). Retrograde tracing showed labelled cord (both rostrally to C4 and caudally to S4) and brainstem neurons. Additional tracing is underway to assess axonal growth from graft into caudal host cord. In sum, MP improves axonal regeneration from both cord and brainstem neurons into SC grafts, possibly by reducing secondary injury of cord adjacent to graft. (NIH NS09923, NS28059 and The Miami Project; XMM is Heumann Int. Scholar.)


We previously demonstrated that Schwann cells (SCs) in semipermeable guidance channels promote regeneration of propriospinal but not supraspinal axons in adult rat spinal cord transected at T8. Here, we tested whether known brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) could stimulate supraspinal axonal regeneration in this model. SCs were purified in culture from adult rat lumbar, caudally suspended with Matrigel and seeded into PAN/PVC channels (Soc. Neurosci. Abstr. 18:1479, 1992). BDNF and NT-3 were delivered together into the capped caudal end of the channel by a miniature mini-pump (18 ml/min) for the first 14 of 30 days tested. Controls received vehicle solution. The spinal cord was transected at T8 and T9-11 segments were removed. The rostral cord stump was inserted 1 mm into the channel. One month after grafting, a mean of 1523 myelinated axons was present in SC/BDNF grafts, twice as many as in SC/vehicle grafts. Brainstem serotonergic axons regenerated into the SC/NT-3 grafts for at least 5 mm, but not in control grafts. When Fast Blue was injected at the channel midpoint, on average, 92 retrogradely labeled neurons were found in 10 specific regions of the brainstem with the highest labeling (67%) in vestibular nuclei. Labeled cells were also present throughout the rostral cord. Thus, regeneration of some neural populations was promoted in a spinal cord transection can be recruited by combinations of trophic factors and a favorable cellular substrate. [Regeneron Pharmaceuticals generously provided neurotrophins and Dr. F. Aeberscher (Lausanne), channels; supported by NIH NS 28059 and NS 09923 and The Miami Project. XMM is the Heumann Int. Scholar.]

EMBRYONIC CNS TRANSPLANTS ASSIST ADULT DORSAL ROOTS TO FORM SYNAPSES IN HOST SPINAL CORD. Y. Itoh*, F. Morii*, T. Supawara*, M. Kowada* and A. Teissier†


Adult dorsal root axons regenerate into embryonic spinal cord transplants and form contacts with transplant endfeet. Stimulating properties of the endfeet are due to electrical stimulation of regenerated dorsal root axons. It is unknown whether embryonic CNS transplants also assist synapse formation by adult dorsal roots regenerated into host spinal cord. Adult Sprague-Dawley rats received intraspinal transplants of E14 spinal cord into left dorsal quadrant cavities in the lumbar enlargement. The severed L4 or L5 dorsal root was sandwiched between the transplant and host spinal cord. Three to 12 months later sagittal sections were processed for calculations of gene-related peptide (CRGP) immunohistochemistry. CRGPeptide immunoreactivity was observed in both cells and endfeet of the E14 M CGRP-labeled axons regenerated into host spinal cord and some extended into the motoneuron pool. The area fraction occupied by regenerating axons in C4 CRGP-labeled transplant was statistically larger than in control rats without transplants. CCRGP-labeled axons formed synaptic terminals within host spinal cord; most were axo-dendritic. Embryonic CNS transplants therefore mediate permanent synapse formation by adult dorsal root axons in host spinal cord and may provide a milieu that promotes reconstruction of interrupted spinal reflex arcs.
458.11

INTERSPINAL WIRING OF THE TRANSCANTED RAT SPINAL COLUMN. Hinchin Cheng, Feng-Lee Huang* and Lara Olson. Department of Neurosciences, Karolinska Institute, Stockholm, Sweden, and Institute of Neuroscience, National Yang-Ming Medical College, Taipei, Taiwan.

Shortening of the spinal column has been regarded as one possible method to obtain cord-to-cord apposition after total transection of the spinal column. However, to further improve regenerative possibilities, the problems of inconsistent bony fusion and cyst formations within the junctions must be resolved. To modify the method of de Medinaceli on the rat thoracic spine, we attempted several strategies to achieve better interspinal fixation after spondylectomy and transsection, including transpedicular miniwires, wiring of the transverse processes, and wiring of the posterior spinal processes. A dynamic model into single fibers was created and compared with the cut ends of the spinal cord by means of adjustable fixation devices to permit swelling and shrinkage of the stumps. The data were also obtained following suppressive wiring of partial spinal processes. Preliminary results support that the best regeneration, as indicated by regrowth of 5-HT fibers below the level of transection, was obtained following suppressive wiring of partial spinal processes. In this group, the distance between two spinal cord stump approximations to spinal levels was inconsistent. To solve the problem of this situation, the MSO spicule was loaded with the hypothermic suspension, and we attempted to use the spinal cord stump approximations to spinal levels. These observations suggest that gap junctions between Schwann cells may be important for both normal function of peripheral nerves and their responses to injury.

458.13


Partial nerve defects are a challenging clinical problem. In this study we examined the regeneration process in partially transected rat sciatic nerve repaired with the previously established silicone chamber model. The tibial nerve fascicle was transected and a 10 mm segment was removed. The tibial proximal and distal stumps together with the intact peroneal fascicle were enwrapped into a silicone tube leaving a 10 mm defect between the two tibial stumps. The regenerated nerve was examined by immunocytochemistry and light microscopy after 7, 16, 28 or 42 days. A cellular fibrin matrix, spanning the proximal and distal stumps of the tibial fascicle and surrounding the intact peroneal fascicle, was formed within one week. This cellular matrix was then invaded by non-neuronal cells and regenerating axons. Regeneration in nerves with a partial defect was more advanced with respect to ingrowth vasculature, Schwann cells, axons and myelination as compared to a totally transected nerve with a 10 mm gap repaired with a silicone chamber. The results suggest that a partial nerve defect could be repaired using the silicone chamber technique. It opens a perspective to solve the clinical problem of repairing a partial nerve defect when neither direct suture nor nerve grafting is applicable.

458.14


The silicone tube technique was used to repair transected median (two cases) or ulnar nerves (one case) at the distal forearm level in three patients. In all three cases the nerve stumps were approximated with a silicone tube of such a dimension that would not cause compression of the nerve and leaving a gap of 3-5 mm between the two nerve ends. Three years after nerve repair the patients were examined including sensory evaluation and assessment of muscle contraction force. In two cases the tubes had to be removed two years (median nerve) or three years (ulnar nerve) after surgery. The case with the shortest type of minor local discomfort. At the time of exploration the former gap was bridged by a smooth continuous nerve structure of the same diameter as the adjacent nerve trunk. There were no signs of neuraoma formation. In the median nerve case there were excellent motor recovery of the thenar muscles. Two point discrimination (2PD) was < 6 mm (12 year old patient) and 8-10 mm (21 year old patient) respectively. In the ulnar nerve case (21 year old patient) the first dorsal interosseus muscle was almost normal in size and the strength remarkably good. 2PD was 6 mm. These three cases have demonstrated that the principle of using the tube technique in humans is feasible. The results have inspired us to conduct a randomized, prospective clinical study of the tube technique versus the conventional technique for repair of median or ulnar nerves at the distal forearm level.

**Neurofibrogya and Myelin III**

459.1


Glutamine synthetase localized in astrocytes utilizes glutamate and ammonia. We tested the hypothesis that astrocyte enlargement associated with hyperammonemia can be reduced by inhibition of glutamine synthetase with methionine sulfoximine (MSO). Pentobarbital-anesthetized rats were pretreated with either vehicle, MSO (150 mg/kg, i.p.), or buthionine sulfoximine (BSO; 88 mg/kg, i.p.), an analogue of MSO that does not inhibit glutamine synthetase. Infusion of ammonia acetate for 6 hours increased plasma ammonia levels from 5.8 to 533 μM. Control astrocytes had marked enlargement of all cytoplasmic areas with increased numbers of mitochondria, rough and smooth endoplasmic reticulum, and glycogen. Enlargement of small astrocytic processes in neuropil and perverscular endfoot was reduced by MSO but not BSO pretreatment despite elevated ammonia levels. Nuclear diameter in hyperammonemic rats treated with vehicle (7.9±0.73 μm; ±SD) was greater than in those pretreated with MSO (7.34±0.94 μm) or in controls receiving sodium acetate (6.79±0.9 μm). These data are consistent with the hypothesis that ammonia-induced astrocyte hypertrophy that occurs during acute hyperammonemia is related to glutamine accumulation and synthesis localized in astrocytes. (Supported by NS22757).

459.2

NITRATE EXCHANGE CHARACTERIZED AS NHE-1 IN RAT HIPPOCAMPAK ASTROCYTES. John P. Panagopoulos1 and Christopher A. Pappas2. Departments of Internal Medicine1 and Neurology2, Yale University School of Medicine, New Haven, CT 06510.

Precise regulation of intracellular pH (pH(i)) in astrocytes is essential for proper function and modulation of the surrounding environment. The Na+/H+ exchange (NHE) is an integral membrane protein which mediates 1:1 electroneutral countertransport of Na+ for H+. Four distinct isoforms (NHE's 1-4) have been reported with differing localizations and physiological properties. NHE-1, an amiloride-sensitive (50μM-10μM) isoform mediating various cell functions such as growth and proliferation, has been found to be expressed in all mammalian cell types examined. NHE-1's 2-4 are less well characterized with a more restricted pattern of localization, which includes brain for NHE-2 and 3, and is known to be amiloride resistant (IC50 > 50μM). We examined NHE-1 expression in primary cultures of rat hippocampal astrocytes. pH(i) recovery from an acid load in the absence of CoCl2 was assessed using BCECF. Rate of recovery from an acid load measured at a pH(i) of 6.7 was 0.193 ± 0.03 pH units/min. and was completely inhibited by 50μM of amiloride (IC50 = 3.18 ± 0.47μM). Northern blot performed under low stringency, using full-length NHE-1 showed expression of a single isoform (~4.8 kb) consistent with NHE-1. Interestingly, application 50μM EIPA, a highly potent amiloride analog, did not inhibit recovery from an acid load and caused a reversible alkalization when applied to resting cells. (Supported by NS 15589 and CT Heart Association).
459.3
RAT HIPOPCAMPAL ASTROCYTES EXHIBIT ELECTROGENIC SODIUM-BICARBONATE COTRANSPORT.
Edward R. O'Connor*, Harald Sontheimer and Bruce R. Ransom.
Dept. of Neurology, Yale School of Medicine, New Haven, CT 06510
Using whole-cell patch-clamp recordings we studied expression of electrogenic Na+/H+ antiport in primary cultures of hippocampal astrocytes derived from newborn rats were studied after 10 days in culture. Application of 25 mM HC03- at a constant pH7,4 to astrocytes bathed in nominally HC03- free solution, produced a reversible change in membrane potentials ranging from +5 to +31 mV (Avc ± s. D. = +11.8±34 mV). The size of the HC03- induced hyperpolarization was strongly related to the cell's initial resting membrane potential; cells with more negative resting potentials had smaller responses. The HC03- induced change in membrane potential was dependent on extracellular Na+, blocked by the dibutyl cyclic dihydro and, of extracellular calcium. Voltage-clamp recording demonstrated that HC03- induced hyperpolarization was caused by outward currents averaging 35±10 pA (holding potential = -90 mV). The reversal potential of the HC03- induced current was between -80 to -90 mV. Based on the reversal potential of the HC03- induced response, and knowledge of the transmembrane gradients for HC03- and Na+, it was calculated that the transporter has an apparent HC03-/Na+ stoichiometry of 2:1. These findings indicate that hippocampal astrocytes express electrogenic Na+/HC03- cotransport. This transporter may play an important role in regulation of intracellular pH, depolarization-induced alkalization and intracellular Na+ homoeostasis. (Supported by NIH grants NS 09542 to ERO and 15599 to BRR).

459.4
ION CHANNELS AND GLIAL PROLIFERATION: II. Role of K+ channels, [Ca2+]i and [pH].
Harald Sontheimer and Christopher A. Pappas
Department of Neurology, Yale University School of Medicine, New Haven CT 06510
Astrocyte proliferation was studied in primary cultures of rat spinal cord to search for factors that might influence glial proliferation. Primary cultures of hippocampal astrocytes derived from newborn rats were studied after 10 days in culture. Application of 25 mM HC03- at a constant pH7.4 to astrocytes bathed in nominally HC03- free solution, produced a reversible change in membrane potentials ranging from +31 to +5 mV (Ave ± s. D. = +11.8±34 mV). The size of the HC03- induced hyperpolarization was strongly related to the cell's initial resting membrane potential; cells with more negative resting potentials had smaller responses. The HC03- induced change in membrane potential was dependent on extracellular Na+, blocked by the dibutyl cyclic dihydro and, of extracellular calcium. Voltage-clamp recording demonstrated that HC03- induced hyperpolarization was caused by outward currents averaging 35±10 pA (holding potential = -90 mV). The reversal potential of the HC03- induced current was between -80 to -90 mV. Based on the reversal potential of the HC03- induced response, and knowledge of the transmembrane gradients for HC03- and Na+, it was calculated that the transporter has an apparent HC03-/Na+ stoichiometry of 2:1. These findings indicate that hippocampal astrocytes express electrogenic Na+/HC03- cotransport. This transporter may play an important role in regulation of intracellular pH, depolarization-induced alkalization and intracellular Na+ homoeostasis. (Supported by NIH grants NS 09542 to ERO and 15599 to BRR).

459.5
ION CHANNELS AND GLIAL PROLIFERATION: II. Role of K+ channels, [Ca2+]i and [pH].
Harald Sontheimer
Interdepartmental Neuroscience Program and Dept. Neurology, Yale University School of Medicine, New Haven CT 06510.

Unlike neurons, glial cells retain the ability to proliferate postnatally and can divide rapidly to form astrocytomas under neoplastic conditions. The precise control of factors involved in proliferation is poorly understood. We studied possible role of K+ channels in regulating proliferation by comparing properties of spinal cord astrocytes, rat C6 glioma cells, and human astrocytoma cells in culture. To determine whether chronic exposure to ion channel blockers alters proliferation, control and sister cultures were grown in the presence or absence of channel-blocking agents, and cell proliferation determined using [3H]-thymidine incorporation as a quantitative measure of DNA synthesis. Application of 4-aminopyridine (2mM), Ba2+ (1mM), Ca2+ (5mM), and TEA (5mM), in concentrations to block glial K+ channels, significantly reduced [3H]-thymidine incorporation in astrocytes, but failed to alter proliferation of neoplastic cells. The phorbol ester PMA (100mM), a known mitogen, increased proliferation in normal astrocytes by 33%, but reduced proliferation in neoplastic cells by up to 50%. As K+ channel block should result in depolarization, we examined the potential interdependence of resting potential and proliferation. Astrocytes in which proliferation was inhibited by cyanide arsenite (ARA-C) exhibited 20-30% more hyperpolarized resting potentials than untreated, proliferating, sister cultures. Similar treatment of neoplastic cells resulted in changes in Vm of the same magnitude, but towards more depolarized potentials. These differences suggest that proliferation is regulated differently in normal and neoplastic glial cells. We are presently investigating the possibility that differences in steadystate pH between control and growth-inhibited cells play a role in limiting ion channel expression, membrane potential, and glial proliferation.

459.7
Division of Neurosurgery and Dept. of Pharmacology/Toxicology, Albany Medical College, Albany, New York 12208
In pathological conditions astrocytes are known to swell markedly and one of the effectors thought to be responsible is an increase in [K+]. Astrocytes are well-known, both in culture and in vivo, to take up the excitatory amino acid glutamate and to release it in a specific manner. Mechanisms of release involving K+-dependent reversal of this transporter and/or cell swelling were studied. Primary astrocyte cultures, from P1 rat cerebral cortex, were exposed to high K+ and the release of [3H]-D-Aspartate was measured. A twenty minute exposure to 100mM K+ resulted in a biphasic release of [3H]-D-Aspartate, an initial transient release response followed by a slower sustained release. L-644,711, an anion transport blocker, blocked the slower release component but had no effect on the initial release component. The initial release component can be enhanced by increasing [Na+] by treating cells with ouabain; which had no effect on the second release component. The initial component seemed more sensitive to increased [K+], while the second component required >100mM K+.
We propose that the initial release is caused by reversal of the uptake transporter and the second caused by a Na+-insensitive swelling induced anion transport process. (Supported by NS 30303)
GLUTAMATE MEDIATES ASTROCYTE-NEURON SIGNALING IN CORTICAL CULTURES. V. Pappas, S. T. Berger, F. Lui, J. Keifer.
459.11 RESPONSE OF ASTROCYTIC GAP JUNCTIONS FOLLOWING NEURONAL DAMAGE IN RAT BRAIN. J. Nagy, M.Z. Houssa, P.A.Y. O'Doherty, L. Murphy, E.L. Hertberg and M.A. Seshwall. Department of Pharmacology, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, CANADA R3E 0W3

The presence of multiple pathways for increasing [Ca2+]j within a single astrocyte suggests the possibility of integration of multiple stimuli.
NEUROGLIA

646.1


Neurotransmitters, growth factors and cytokines have been shown to mediate neuronal-cytochrome communication. Astrocytes are shown to exhibit receptors for most of these mediators. Activation of receptors by agonists results in a cascade of intracellular events, including the stimulation of calcium influx and subsequent phosphorylation of substrate proteins. To study the regulation of astrocytic functions, we analyzed intracellular target phosphophosphoryts of extracellular signals, in cultured astrocytes using 2-dimensional polyacrylamide gel electrophoresis. Previously, we described a novel substrate of protein kinase C (PKC) enriched in astrocytes, PEA-15 (J. Biol. Chem. 268:5911-5920). Recent RFLP studies performed both in vivo and in vitro, followed by microsequencing, suggested that PEA-15 is also a substrate for calcium-calmodulin-dependent protein kinase II (CaMkII) at a seril residue different from the PKC site. Indirect immunofluorescent studies using antibodies raised against synthetic peptides corresponding to the two phosphorylated sites, demonstrated that PEA-15 is colocalized with microtubules in cultured astrocytes. To determine which form(s) of the protein associate preferentially with tubulins, polymerized tubulin was separated from unpolymerized tubulin by using solubilizing triton-X 100. Analysis of the microtubule-associated fraction suggests that both PKC and CaMKII phosphorylation of PEA-15 may be involved in regulating the binding of the protein to microtubules.

646.2

REGIONAL AND FOCAL ALTERATIONS IN NEUROFILAMENT AND TUBULIN IMMUNOREACTIVITY IN INJURED RAT BRAIN. K. E. Saatman and T. K. McIntosh*. Dept. of Neurosurgery, Univ. of Pennsylvania, Philadelphia, PA 19104.

Axonal injury resulting from rapid deformation of the brain is often characterized by the presence of notochordal axonal swellings (club-like endings) (terminal clubs) and axons with regions of enlarged diameter. These pathological changes in axonal morphology have been described clinically, in cases of diffuse axonal injury, and in animal models of acceleration and fluid percussion brain injury. We have used antibodies to tubulin, tyrosinated tubulin, and both 68 kD and 160 kD neuron-specific (NF) subunits to examine axonal swelling and terminal clubbing in the rat brain following fluid percussion brain injury. In addition, regional alterations in the immunoreactivity (IR) of these cytoskeletal proteins were investigated. Adult male Sprague-Dawley rats were anesthetized (sodium pentobarbital, 50 mg/kg) and subjected to lateral fluid percussion brain injury of moderate severity (2-4 atm). Animals were sacrificed at 2, 3, or 7 days post-injury. Cortical neurons surrounding the injury site showed decreased tubulin and NF IR and disruption of their normal radial alignment when compared to the contralateral cortex and to sham-injured animals. Decreased tubulin IR was also seen in the CA3 region at all time points. At 3 days, typical axonal swellings and terminal clubs were readily visible in injured rats, using NF antibody labeling. Populations of these damaged axons were seen in the ipsilateral subcortical white matter, cortex, and thalamus. While these focal axonal swellings could be easily located due to their intense NF IR, neither tubulin antibody used in this study showed intense labelling at the regions of axonal damage. (Supported, in part, by NS26818 and NS08803.)

646.4

A MICROTRIT TER PLATE FORMAT FOR THE MEASUREMENT OF TAxOId-INDUCED POLYMERIZATION OF TUBULIN. F. E. Macdonald, S. Blumenthal-Z, F. Gu, K. Nyberg and B. S. Glasser*, PHYTOPharmaceuticals, Inc., San Carlos, CA 94070 and S. Mudumba, Department of Pharmacology, University of the Pacific, Stockton, CA 95211.

We describe a 96-well plate assay for the measurement of tubulin polymerization based on the method described by Gaskin et al. (1994). Tubulin was purified from fresh bovine brain by two cycles of assembly and disassembly induced by heat, glycerol and OTP, followed by a third cycle with assembly induced by DMSO. For the assay (200ul), tubulin is diluted (0.1 M PIPES, 1Mm EGTA, 1Mm MgSO4). Prior to tubulin addition, each microtiter well receives 100ul of sample or reference compound in 10%DMSO for 30 min. Optimization concentration is 0.75mg/ml. Polymerization is monitored kinetically at 540nm and at 374nm. Thermomax plate reader (Molecular Devices Corp., Menlo Park, CA). The OD340 correlates with compound concentration until saturation of binding sites. Compound potency is expressed as the concentration of compound causing a response equal to 50% of that observed at saturation (IC50). Results in the Table show good reproducibility. Compounds promoting the dissociation of tubulin/NGI, a scaffold-like protein that prevents GTP off, inhibit taxol-induced polymerization in a dose-dependent manner. This assay may be useful for the characterization of compounds that affect tubulin assembly and disassembly such as specific microtubule modulators.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>IC50 (nM)</th>
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<tbody>
<tr>
<td>Taxol</td>
<td>12</td>
</tr>
<tr>
<td>Cephalomamine</td>
<td>3</td>
</tr>
<tr>
<td>7-epi-10-deacetyltaxol</td>
<td>3</td>
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460.5 PHOSPHORYLATION AND CONFORMATIONAL CHANGES OF MAP2 DURING CAT BRAIN DEVELOPMENT
B.M. Riederer, E. Dibrovsky, P. Dibrov, V. Viliksci.
Institute of Anatomy, University of Lausanne, Lausanne, Switzerland; Institute of Molecular Genetics, Prague, Czech Republic.
Molecule-associated protein 2 (MAP2) is essential for the stability of microtubules and for dendritic growth. In this study five monoclonal antibodies were used to analyze MAP2 during brain development. Antibody AP18 was the only one directed against a phosphorylation-dependent epitope on MAP2b and c. Dephosphorylation with alkaline phosphatase abolished most immunoreactivity with AP18 and indicated that MAP2 is phosphorylated throughout postnatal development, possibly by a calcium-dependent kinase. The AP16 epitope was less susceptible to dephosphorylation in larger apical dendrites of cortical pyramidal cells and in some neurons of the upper layers of visual cortex. It is suggested that the AP18 epitope is less accessible to alkaline phosphatase, and that MAP2 conformation changes may differ within parts of neurons. Three antibodies, AP14, MT01, MT02, were directed against the central region of MAP2b. An immunocytochemical comparison revealed differences in the cellular distribution during early postnatal development of cat cerebellum, while at later ages, most antibodies stained similar neuronal elements. Antibody MT02 stained at early stages cell bodies and dendrites in cerebellar cortex and cerebellum. Within the first postnatal month immunoreactivity became restricted to the distal parts of apical dendrites of pyramidal cells but staining was absent from perikarya and finer basal dendrites, and no immunostaining was found in cerebellum. MT02 immunoreactivity is independent of phosphorylation. It is suggested that immunocytochemical detection of MAP2 depends on its conformation, posttranslational modifications such as phosphorylation, or masking of sites by other proteins. Supported by grants of the Swiss National Science Foundation 31-3447.92 and 37TPRJO38808.

460.6 REGULATION OF NEURONAL CYTOSKELETAL PHOSPHORYLATION BY CATION FLUX
M. Mars**, and D.J. Firek, Department of Neurology and ORED, University of Michigan, Ann Arbor, MI 48105 and University of Lausanne, Lausanne, Switzerland.
We studied the state of cytoskeletal terminal phosphorylation of neurofilament (NF) proteins, and of the microtubule-associated proteins in rat brain aggregates in culture. Cultures containing rat telencephalic neurons and glia in defined medium were grown for 4 weeks until myelination occurred. The cultures were then treated with 300 μM veratridine or calcium ions in order to trigger Na+ influx. We used Western blot with antibodies BS1-31 (Sterneberg Meyer) and N-14 (Boehringer Mannheim) to detect phosphorylated NFs, and AB 1991 (Chemicon) to detect MAP tau-1 independent of phosphorylation. Antibody tau-1 (Boehringer-Mannheim) was used to detect tau.
Beginning at 2 hrs of veratridine treatment, there was a marked decrease in phosphorylated NFs, with no change in total NF content, although a shift in mobility of total NFs detected by AB1991, consistent with dephosphorylation of NF-H. Veratridine treatment also resulted in multiple bands with more rapid electrophoretic mobility detected with the tau-1 antibody, consistent with dephosphorylation of tau.
Prolonged treatment with veratridine led to a decrease in total content of a variety of calpain substrates (NF, tau, GAP-43 and calcium) without affecting the level of other proteins that are not calpain substrates (membrane Na-K-ATPase al subunit).
These results suggest that sodium entry through voltage gated channels may regulate the dephosphorylation of cytoskeletal proteins while prolonged treatment may result in Ca2+ entry and activation of calpains.
Supported by grants from the Zyma Foundation and the NIH.

460.7 PROPOFOL INDUCES NO CHANGES IN ACTIN IN THE CYTOSKELETON WHEN INTRACELLULAR CALCIUM IS DEPLETED
By MARTA A. VLAP and PIETER A. G. J. Joosse and A. R. Sengers, Departments of Anaesthesiology and Cell Biology, Faculty of Health Sciences, University Hospital, S-581 85 Linkoping, Sweden.
The site of action of the intravenous anesthetic drug propofol (Diprivan®) is still uncertain. The intracellular action of propofol is studied on primary cultures of neurons from rat. Cytosolic free calcium changes in neurons were studied using Fura2 and a single-cell microfluorometric method. Fluorescence microscopic analysis enabled the body of the organism of activity. An increase in the cytosolic free calcium concentration (Ca2+free) amounting to 24±3E3M was seen shortly after addition of 3μg m.l-1 of propofol. When neurons were depleted in intracellular calcium by the presence of MAPTMA and stimulated by 3μg propofol no changes were seen in the actin organisation of cytoskeleton, as revealed by a fluorescence microscopy. Only 18 of 306 cells exposed to 3μg m.l-1 of propofol exhibited changes in the morphology. Of 250 untreated cells that served as controls, 21 showed morphological changes.
Propofol in concentration exceeding or equivalent clinical relevant concentration induces changes in the morphology of cultured neurons from the rat. These changes are a consequence of the increase in cytosolic free calcium concentration seen after stimulation with propofol. Evidence of this is that no change in actin organisation were observed when intracellular calcium was depleted.

460.9 Vilip 22 kD neuronal EF-hand Ca2+-binding protein from chick brain: regulation of its interaction with intracellular target molecules. K.H. Braunsteiner, R. E. Lent and E. D. Gundelfinger*, Federal Institute for Neurobiology (IFN), Dept. of Neurochem./Mol. biology, P.O. Box 1860, D-39008 Magdeburg, F.R.G.
Vilip (Vilip: Vila-lipin) (Lent et al., 1992, Mol. Brain Res., 15, 133-140) is a member of a new family of neuronal Ca2+-binding proteins, such as vila, recoverina-modal or frequenin. The function of these proteins remains unclear.
Vilip is expressed by a subset of neurons in the brain and retina. The molecule is localized in the cell body, dendrites and axons, and seems to be associated with the cortical cytoskeleton. At least one of the four EF-hand structures of Vilip is able to bind Ca2+-ions, as revealed by mobility shift analysis. The molecule is able to interact with membrane- and cytoskeletal cellular fractions in a Ca2+-dependent manner. In overlay assays several EF-hand containing binding partners for Vilip have been identified. One of them is actin, a main component of the cortical cytoskeleton. Several potential phosphorylation sites exist in the EF-hand structures of Vilip. This raises the possibility that the ability of Vilip to bind Ca2+-ions and to interact with intracellular target molecules in a Ca2+-dependent manner might be regulated. These results suggest that Vilip may act as a Ca2+-effectomolecule. It may transmit Ca2+-signals to the cytoskeleton, thereby affecting processes, such as neurotransmitter release, cell outgrowth or synaptic plasticity, which are dependent on the dynamics of the cytoskeleton. This work is supported by DFG.

460.8 CORTICAL INTERNEURONS EXPRESSING CALCIUM BINDING PROTEINS HAVE AN ERYTHROCYTE-RELATED MEMBRANE CYTOSKELETON. M. R. Celesia1, R. M. Riederer, S. Langer,2 1Department of Histology and general Embryology, University of Fribourg, CH-1705 Fribourg; 2Institute of Anatomy, University of Lausanne, Lausanne; Howard Hughes Medical Institute and Dept. of Biochemistry, Duke University, North Carolina USA.
Cortical interneurons have a non-pyramidal shape, receive their inputs mainly on the cell body and proximal dendrites, express a calcium-binding protein of the EF-hand family and are often surrounded by the "perineuronal net", a specialized extracellular matrix containing e.g. restrictin/janusin. We have found by immunohistochemistry that cortical interneurons immunoreactive for the calcium-binding protein parvalbumin, calcium-dependent 28k and calrein express the red blood cell isofrom of spectrin and ankyrin (βg spectrin brain spectrin 240/235 B or β5 Splb and ankyrin). The immunolabelling for the two calcium associated proteins in each nerve cell is restricted to the cortical portion of the cytoplasm, whereas the calcium-binding proteins occur dispersed in the whole cytoplasm. Ankyrin and βg spectrin immunolabelling is reticulated, whereas the ankyrin staining is punctuated. The study of the distribution of ankyrin and βg spectrin in relationship to restrictin/janusin reveals that the membrane cytoskeletal proteins are in register with the extracellular matrix protein. We suspect that calcium-binding proteins interact with the proteins of the membrane cytoskeleton, thus modulating the lateral mobility of integral membrane proteins and the adhesion of interneurons to the extracellular matrix.

460.10 SORTING SIGNALS FOR TRANSFERRIN RECEPTOR AND RETROVIRUS PROTEINS IN AXONAL AND DENDRITIC TRANSPORT. K. Kristensson*, E. Weeluccius and W. Carolff, Karolinska Institutet, Dept. of Neuroscience; S-17177 Stockholm, Sweden, Dept. of molecular Biology, Karolinska Institutet, Riddings, Sweden.
Rat embryonic dorsal root ganglia, spinal cord and hippocampal neurons in culture were infected with recombinant Semliki forest virus (SFV) containing the genes for the human transferrin receptor, the envelope or macrophage-activating proteins of human immunodeficiency virus type 1 (HIV-1) or Kolesney murine leukemia (Mo-MLV) virus. All nerve cell lines expressed the human transferrin receptor in all cell bodies, but not in their axons. In spinal cord and hippocampal neurons the receptor also occurred in the dendrites. A mutant form of the receptor, in which the amino acids 6-53 in the cytoplasmic tail had been deleted, appeared both in axons and dendrites. HIV-1 envelope protein was also exclusively localized to the dendrites, while the gag protein appeared both in axons and dendrites. Co-expression of the envelope and the gag proteins resulted in a dendritic localisation of the latter, indicating that the envelope protein can interact with and determine the intracellular sorting of the gag protein. Similarly, the Mo-MLV gag appeared in both axons and dendrites, but only in dendrites when co-expressed with the envelope protein. Heterologous co-expression of HIV-1 envelope and Mo-MLV gag proteins and vice versa did not influence the distribution of the gag proteins.
DENDRITE TRANSPORT OF NEURAL BCI RNA IN SYMPATHETIC NEURONS IN CULTURE. L. Mastromatteo, S. Pernini, D. Higgins. H. Tiedge. 1Fishberg, 2Neurobiology, Mount Sinai School of Medicine, New York, NY 10029. 2Department of Pharmacology, SUNY at Buffalo, Buffalo, NY 14214.

Extrastratal protein synthesis in postsynaptic dendritic domains may contribute significantly to long-term synaptic plasticity of nerve cells [see Steward and Banker (1992), Trends Neurosci. 15, 180-186, for review]. In this model, selected RNAs would have to be preferentially targeted to dendritic microdomains. Here, the mechanism of dendritic RNA targeting and transport has remained elusive.

We have analyzed the dendritic targeting competence of neural BCI RNA. This short, non-translatable RNA has been identified in somatomedendritic domains of neurons (Tiedge et al. 1991), Proc. Natl. Acad. Sci. USA 88, 2093-2097; where it may participate in transport- and/or translation-related processes. We have generated various radiolabeled BCI sequences, including full length BCI RNA as well as 5’ and 3’ segments, and microinjected them into somata of rat sympathetic primary culture. We observed a significant and specific accumulation of full length BCI RNA in dendrites. In contrast, injected small nuclear RNAs, such as U4 and U6 RNAs, distributed to somata and muscles. Even when antibodies to any significant extent. Transport of BCI RNA was rapid: distal dendritic tips were reached in less than 2 h. The various structural subdomains in BCI RNA showed significant differences in their dendritic targeting potentials. These results support the notion that BCI RNA contains specific signaling elements that direct its sorting and targeting to dendritic domains.

Supported by NSF grant IBN-921949 to HT.)

SYNAPTIC STRUCTURE AND FUNCTION III

COSYNEURON STRUCTURE IN POSTSYNAPTIC MEMBRANE TARGETING: AXON AND DENDRITE. C.J. KRAMER, F. JARMAN, F. SIEWERT, J. O’ROURKE, J. MOSCA, C. DAVIS, P. PARADISO, N. FOSTER, H. TIEDE. 1Fishberg, 2Neurobiology, Mount Sinai School of Medicine, New York, NY 10029. 2Department of Pharmacology, SUNY at Buffalo, Buffalo, NY 14214.

DENDRITE TRANSPORT OF NEURAL BCI RNA IN SYMPATHETIC NEURONS IN CULTURE. L. Mastromatteo, S. Pernini, D. Higgins. H. Tiedge. 1Fishberg, 2Neurobiology, Mount Sinai School of Medicine, New York, NY 10029. 2Department of Pharmacology, SUNY at Buffalo, Buffalo, NY 14214.

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Supported by NSF grant IBN-921949 to HT.)

SYNAPTIC STRUCTURE AND FUNCTION III

PSD-95 is a prominent, highly-enriched protein in postsynaptic density fractions prepared from rat brain. PSD-95 has an apparent molecular weight of 95,000 and is enriched in the PSD fraction where it comprises about 1% of total protein. It was purified from the postsynaptic density fraction, and tyrosine phosphorylated and dually labeled with a combination of the membrane-impermeable dyes di-4-ANEPPS and FM1-43. The detergent solubilized protein was purified by precipitation with ammonium sulfate and by ammonium acetate. We have purified a recombinant protein expressing a C-terminal 6X-His tag. We present a preliminary characterization of purified recombinant PSD-95.


We have identified a novel protein, termed PSD-UP180, from the postsynaptic density (PSD) fraction of rat brain. PSD-UP180 has an apparent molecular weight of 180 kDa, and is enriched in the PSD fraction where it comprises about 1% of total protein. It was purified from the postsynaptic density fraction, and tyrosine phosphorylated and dually labeled with a combination of the membrane-impermeable dyes di-4-ANEPPS and FM1-43. The detergent solubilized protein was purified by precipitation with ammonium sulfate and by ammonium acetate. We have purified a recombinant protein expressing a C-terminal 6X-His tag. We present a preliminary characterization of purified recombinant PSD-95.


Recent evidence showing high levels of protein tyrosine kinase (PTK) activity in adult rat brain was shown. Evidence shows that protein tyrosine kinase (PTK) activity is crucial for synaptic function, we examined this activity in the postsynaptic density fraction of rat brain. Our results suggest that there were 2 PTKs (M.W = 166, 90, 66, 50 kDa) present in the cortical PSD of adult rat brain. Results of phosphorylation analysis and confidence PTNs of these kinase. Recently, Csk, designated as Csk-like type, was found to be predominantly expressed in the brain. Similarity in molecular weight and unique localization in the brain suggest that Csk and the 50 kDa PTK in the PSD may be similar or identical. Indeed, we found that 50 kDa PTK co-precipitated with anti-Csk antibodies in brains sections as well as PTNs. Consequently, we observed the 50 kDa PTK in the PSD Csk-like PTK, and have employed the antisera to Csk to begin characterizing the enzyme in synaptosomes. Our results, obtained by Western blot analysis, suggest that the Csk-like PTK was selectively localized to the PSD. Further, the protein was differentially expressed in the PSD isolated from hippocampus, cerebral cortex, cerebellum and olfactory bulb, implying region-specific function. Among the 4 brain regions examined, the PTK was expressed most abundantly in the hippocampus, an area of crucial importance for learning and memory. The identity of the Csk-like PTK remains to be determined. Nonetheless, our present findings suggest that the enzyme may play important roles in synaptic function at the postsynaptic site.

461.8 A MULTITUDE OF SYNAPTOSOMAL PROTEINS POSSESS O-LINKED NACETYLGALACOSAMINE. N. Cole* and G.W. Hart. Dept. of Biochem. and Molecular Genetics, Univ. of Alabama, Birmingham, AL 35294.

Glycosylation of Src/Thr tyrosine kinase substrates N-linked glycoprotein 0-N-acetylgalactosamine (O-GlcNAc) is a dynamic post-translational modification on a variety of nuclear and cytoplasmic proteins. This abundant form of glycosylation often occurs at sites of protein-directed protein kinases and appears to have a reciprocal relationship with protein phosphorylation. A current hypothesis views O-GlcNAc glycosylation as a mediator of reversible association/disassociation of multi-protein complexes.

Recent studies have demonstrated that the neuron-specific phosphoproteins, synapsins, neurofilaments, and now, tau proteins (Arnold et al., 1994, Soc. Neurosci. Abst.), also possess the O-GlcNAc modification. As a first step towards elucidating a role of O-GlcNAc glycosylation in the nervous system, we assessed for the presence of O-GlcNAc in synaptosomes. Galactosyltransferase, a highly specific probe for terminal GlcNAc residues, labeled numerous synaptosomes, ranging from 30-200 kD. More than 60% of the label associated with synaptosomal proteins was associated to PAGase F (removes terminal sugars), but sensitive to alkaline β-elimination (removes O-linked sugars). Analysis of these β-elimination products by gel filtration and Dionex HPLC confirmed that the O-linked sugars originate from single GlcNAc modifications. Taken together these data indicate that O-GlcNAc is abundantly present on synaptosomal proteins.

We also detected O-GlcNAc transferase activity in synaptosomal preparations. This, the dynamic form of glycosylation could play a role in the association/disassociation of multi-protein complexes regulating neurotransmitter release.

461.9 LIPASES AND PHOSPHOLIPASES IN SYNAPTOENEURONOS FROM RAT, MOUSE, AND PIG BRAIN. L.A. Horrocks, H.-C. Yang, and A.A. Farcougl. Dept. Med. Biochem., The Ohio State Univ., Columbus, Ohio 43210.

Lipases (EC 3.1.1.34) and phospholipases (EC 3.1.1.4) are involved in signal transduction and neurodegeneration. Compared to homogenates, synaptosomes from cerebral cortex of rat, mouse, and pig brain exhibit significantly higher activities of triacylglycerol and monoacylglycerol lipase activities and 15 to 20% of the Ca2+-independent phospholipases A2 activities of lipases are higher in pig and mouse brain synaptosomes than in rat brain synaptosome homogenates. Lipase and phospholipase A2 activities of synaptosomal fractions from various animal species were similar. However, the specific Ca2+-independent phospholipase A2 activity, acting on 1-alk-1-enyl-2-acyl-sn-glycerol-3-phosphoethanolamine was higher than that of the Ca2+-independent phospholipase A2 activity, acting on 1-alk-1-enyl-2-acyl-sn-glycerol-3-phosphoethanolamine. The occurrence of lipases and phospholipases in synaptosomes from rat, mouse, and pig brain suggests that these organelles provide a useful model for studying signal transduction systems. Supported by NIH grants NS10165 and NS29441.

461.10 A NOVEL INTERACTION BETWEEN SYNAPTOBREVIN AND SYNAPTOPHYSIN. L. Edelman*, E. Chapman and R. Jahn, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510.

Synaptobrevin and synaptophysin are integral membrane proteins of synaptic vesicles. It has recently been established that synaptobrevin is a key component of the exocytotic fusion complex. In contrast, the function of synaptophysin is still undefined. It has been proposed to form a channel which may play a role in transmitter release.

Here we report about a specific interaction between synaptobrevin and synaptophysin. Using a novel monoclonal antibody for synaptobrevin II, we performed immunoprecipitation experiments with synaptic acetylcholine extracts. With this antibody, synaptobrevin co-immunoprecipitated with syntaxin and SNAP-25, the members of the putative fusion complex. When a monoclonal antibody for synaptophysin was used, synaptophysin but none of the other proteins co-immunoprecipitated. The interaction between synaptobrevin and synaptophysin was further investigated using recombinant synaptobrevin GST fusion proteins immobilized to glutathione beads. Synaptobrevin bound to all isoforms of synaptophysin including phalloidin. Our findings indicate that synaptobrevin participates in at least two independent sets of protein-protein interactions. Thus it is possible that the availability of the v-SNARE synaptobrevin for membrane fusion may be regulated by synaptophysin or synaptophysin-like proteins indicating that synaptophysin may function as an important regulator of exocytosis.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
461.11 PROTEIN-PROTEIN INTERACTIONS BETWEEN ACTIN AND ACONITASE-LIKE PROTEINS IN THE PHOTORECEPTOR TERMINAL. 
E.A. Zastawny*, G.W. Balakrishna*, Department of Biology, Boston College, Chestnut Hill, MA 02167.

Actin binding proteins are important in the dynamic role the actin molecule plays in the architecture of the cellular cytoskeleton. It is thought that many of these actin binding proteins interact with actin by mimicking certain amino acid sequences within the actin molecule. Certain actin binding proteins are found concentrated at the presynaptic terminal of neurons. These proteins function in the regulation of synaptic vesicle exocytosis. We have generated a monoclonal antibody, B16, that labels the presynaptic structure of photoreceptors. Biochemical analysis of tissue homogenates shows that B16 also recognizes four proteins. By use of the recombinant protein, the RBK protein, we have demonstrated binding to homologous actin. Aconitase is found in two forms, mitochondrial, and cytoplasmic. The latter enzyme is capable of being released for translational regulation. Both forms of the enzyme have the citrate to isocitrate activity.

We decided to investigate the relationship between aconitase and actin. Amino acid sequence comparison of aconitase, actin and other actin binding proteins showed that aconitase contains two motifs implicated in the molecular memory of other actin binding proteins. We found that the positive actin binding sites were active by probing western blots of aconitase and aconitase containing homologues with bovine actin (rat-actin). Next to determine if this actin-aconitase interaction was functional we found that aconitase contributes to the polymerisation of actin into a filamentous network in low Ca2+. Aconitase is also recognized by rat-actin and is a monoclonal antibody. These results suggest there may be a functional role as well in physiological processes such as aconitase and other actin binding proteins. (Supported by Boston College REU)

461.12 NEUROCHEMICAL CHARACTERISTICS OF A POSTSYNAPTIC DENSITY (PSD) FRACTION ISOLATED FROM BOVINE RETINA. 

Considerable evidence suggests that the PSD, a disc-shaped proteinaceous structure attached to the inner surface of postsynaptic membrane, play a crucial role in synaptic function. We have previously demonstrated that the molecular characteristics of the PSD differ among different regions of the cerebellum (CTX, cerebellum (CBL), hippocampus (HI) and olfactory bulb (OB)). To further characterize the regional differences of PSD characteristic throughout the brain, we examined the adult bovine retina, a structure richly innervated by both peripheral and central afferents. Bovine CTX-PSD was studied in parallel for comparison. Morphologically, both the CTX-PSD and retina-PSD exhibited a typical disc-like structure. On average, the retina-PSD was shorter in length than the CTX-PSD and appeared to be more sensitive to Triton X-100. A monoamine degergent employed for isolation of the PSD. SDS-PAGE profiles of the CTX-PSD and CTX-PSD were similar qualitatively, but were different quantitatively. Both bovine retina- and CTX-PSD contained several intrinsic protein kinase activities, similar to those found in rodent and canine PSDs. We conclude that bovine retinal PSD may provide a useful model to study the regulation of synaptic molecular architecture in the visual system in response to environmental stimuli.

PRESYNAPTIC MECHANISMS III

462.1 DIRECT RECORDING FROM IDENTIFIED SYNAPTIC TERMINALS IN THE RAT AUDITORY PATHWAY: EXAMINATION OF A GLUTAMATE AUTORECEPTOR. 
J.D. Forster*, M. Barnes-Davies. Department of Cell Physiology & Pharmacology, Leicester University, LE1 9HN, U.K.

We are interested in the mechanism by which presynaptic glutamate receptors mediate transmission at excitatory synapses. A spatial analysis can give indirect information on the spatial probability of transmitter release, but to address the mechanism of such phenomena, direct presynaptic recording is desirable. The small size of most synaptic terminals in the mammalian CNS makes direct recording impossible, so we have developed a slice preparation of the rat brainstem, containing the superior olivary complex, within which a large synaptic terminal called the calyx of Held is localized.

Transverse slices were prepared from 6-12 day-old Lister Hooded rats. Whole-cell single channel recordings were made from the principal neurons in the medial nucleus of the trapezoid body (MNTB). Dual component monomeric EPSCs were evoked by stimulation of the presynaptic axon. A fast component is mediated by AMPA receptors and has an EPSC probability of transmission nearly equal to unity. In the presence of NMDA-receptor antagonists, perfusion of 10-500 nM ACPD or L-AP4 depressed the EPSC by up to 80 % as has been observed in the hippocampus (Forster & Clements, J. Physiol. 429: 1-16, 1990). Analysis of the coefficient of variance for the evoked EPSCs showed that both drugs act at a presynaptic site to depress transmitter release. The putative mGluR antagonist to-methyl-4-carboxyphenylglycine (1 mM) had no effect on the presynaptic depression.

Direct patch recordings from these presynaptic terminals have been achieved, using Lucifer yellow to confirm the identity of the recording site. Under current clamp, the action potential possessed a profound after-hyperpolarization and responded to depolarizing current injection with a train of APs at up to 200 Hz. (25°C). In voltage-clamp a tetradotoxin-sensitive inward current was followed by a rapidly activating outward current which was sensitive to micromolar concentrations of 4-aminopyridine. (Supported by the Wellcome Trust.)

462.2 GABA_A AND METABotropic RECEPTOR AGONISTS ATTENUATE EVOKED EXCITATORY POSTSYNAPTIC CURRENTS IN LATERAL PARABRACHIAL NUCLEUS (LBPN) NEURONS IN VITRO. 
J.A. Zidovitch*, J.E. Essaye & J.J. Marder*. Department of Medicine (Neurology), University of Alberta, Edmonton, Canada.

The LBPN is a major recipient of autonomic information from more caudal regions of the brainstem. Previously, we have reported that postsynaptic excitatory (NMDA & non-NMDA) and inhibitory (GABA_A) type receptors mediate neurotransmission within the LBPN. The present study shows that excitatory postsynaptic currents observed in the LBPN are attenuated by GABA_A and metabotropic receptor agonists. These actions are independent of any apical postsynaptic effect and suggest that the principal transmitters released in the LBPN can negatively influence transmitter liberation via a presynaptic mechanism of action.

LBPN neurons were recorded from 400 μm thick coronal brainstem slices using the whole-cell patch clamp technique. Monosynaptic excitatory postsynaptic currents (EPSCs) were evoked via bipolar electrodes (6.0 Hz; 100 μA; 10-50% placed across the dorsal medial aspect of the LBPN. Bath application of the metabotropic receptor agonist, trans-1-amino-cyclopentane-1,3-dicarboxylate (t-ACPD; 10 μM) reduced EPSC amplitude by 45.9 ± 6.9% (n=9). No concomitant change to the resting current (V_rest >60 mV) or the current observed during voltage ramp (-120 to +20 mV) was observed. The GABA_A agonist, baclofen (BAC; 1 μM), similarly reduced the amplitude of the EPSC by 61.3 ± 5.3% (n=2). Bath application of BAC at this dose did not activate postsynaptic GABA_A receptors as no change was observed in resting current or voltage ramp-generated currents. These results provide evidence for the existence of two receptor-mediated inhibitory intrinsic mechanisms that reduce evoked transmitter release from terminals and thereby influence the excitability of LBPN neurons.

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462.3 PREGNANIC METABOTROPIC GLUTAMATE RECEPTOR REDUCE SYNAPTIC TRANSMISSION IN THE NEONATAL RAT HIPPOCAMPUS. 
T.G. Dunst* and T.C. Foster, Dept. of Psychology, Univ. of Virginia, Charlottesville, VA 22903.

Prenatal modification of synaptic transmission occurs in the developing rat hippocampus. Developmental increases in population and unitary EPSPs are accompanied by decreased paired-pulse facilitation suggesting an increase in the probability of transmitter release. This increase may reflect developmental regulation of autoreceptors. Previous research has shown that the metabotropic glutamate receptor (mGluR) antagonist, trans-ACPD, depresses CA3-CA1 EPSPs in voltage-clamped CA1 neurons in neonatal rats. This experiment investigates possible presynaptic effects of mGluR antagonists on CA1 cells. Whole-cell recordings of CA1 mGluR agonists were recorded from CA1 neurons of 10-15 day-old rats. mGluR agonists were recorded from area CA1 in hippocampal slices taken from male or female rats after the fifth week of gestation. Agonists applied at a concentration of 10 μM were found to reduce AMPA EPSPs by 40% on average. These effects were reduced by 20% after the second week of gestation. These effects were significantly increased in males as compared to females. These results suggest that glutaetatic synaptic transmission, which may mediate physiologically important inputs to the NMDA, can be modulated presynaptically by GABA_A and metabotropic receptors. (Supported by the MRC Canada and the AHFMRI)

462.4 PREGNANIC MODULATIONS OF GLUTAMAMIC NEUROTTRANSMISSION BY GABA_A AND METABotropic RECEPTORS IN THE HORIZONTAL LIMITS OF THE DIAGONAL BAND OF BROCA (hBB) IN VITRO. 

The D8B, a basal forebrain cholinergic nucleus, participates in central autonomic regulation, theta rhythm generation, and memory mechanisms. Previously, we reported that GABA_A and metabotropic receptors mediate excitatory transmission in hBB neurons. In the present study, we examined the mechanism whereby GABA_A and metabotropic receptors modulate glutaetatic neuronal transmission. Whole cell patch clamp recordings were obtained from coronal slices (400μm) of the rat forebrain containing the hBB. Bipolar stimulation electrodes (200 μA duration; 2Hz; range 10-100 Hz) were used to determine voltage ramps (-140 to +40 mV; 20 sec) (n=12). NBOX (10 μM), an AMPA receptor-selective antagonist, also attenuated the EPSP. The metabotropic receptor antagonist trans-ACPD (10 μM) also reversed the changes in resting current or input resistance (n=13). Furthermore, both baclofen and trans-ACPD did not alter postsynaptic AMPA-mediated inward current, thereby confirming their presynaptic locus of action. These results suggest that glutamamatic synaptic transmission, which may mediate physiologically important inputs to the hBB, can be modulated presynaptically by GABA_A and metabotropic receptors.

Supported by the MRC of Canada and the AHFMRI.
A new technique for comparing evoked and spontaneous synaptic transmission at corticostriatal synapses. We have found that spontaneous EPSCs (sEPSCs) in striatal slices are very small and infrequent. Thus, we have developed a new preparation in which we grow cortical explants in dissociated striatal neurons. The cortical explants (250 x 500 μm) extend processes and form synapses with the neighboring striatal cells. In this preparation we can record sEPSCs using whole-cell recordings in the striatal cells. In the presence of TTX, sEPSCs reconstitute EPSCs (meEPSCs). To record evoked EPSCs in the same striatal cells a bipolar electrode placed on the cortical explant is used to stimulate the tissue. 5 μM dNQX blocks meEPSCs, sEPSCs and EPSCs indicating that synaptic transmission in this system is glutamatergic.

The amplitude of evoked EPSCs is inhibited by GABA-A receptor and by adenosine. 5 μM T-ACPD produces a 9.8 ± 3.6% (n=9) potentiation, 50 μM T-ACPD produces a 31.3 ± 5.2% (n=7) inhibition, 75 μM T-ACPD, 46.9 ± 5.2% (n=8) inhibition, and 50 μM adenosine. 43.8 ± 8.6% (n=5) inhibition. Spontaneous transmission is also affected by GABA-A receptor with T-ACPD. 5 μM T-ACPD has no effect on the mean amplitude of meEPSCs and decreases the time between events by 21.8% (n=2), 50 μM T-ACPD produces a 12.3 ± 10.0% (n=7) inhibition of the mean amplitude and increases the time between events by 100 ± 84.9%. 50 μM T-ACPD appears to have no effect on the mean amplitude of meEPSCs (n=3) and increases the time between events by 0 - 35%. We have successfully used this preparation to look at evoked and spontaneous transmission at the same synapse and have shown that GABA-A activation affects both. (Supported by NS 30470)

**PRESYNAPTIC CHARACTERISTICS OF TWO GABA_A IPSCS IN RAT HIPPOCAMPAL CA1 NEURONS. R.A. Peper* & S.D. Gustafson. Departments of Anesthesiology, Madison, WI 53792.**

Acting via a GABA_A autoreceptor, GABA limits its own release during trains of synaptic excitation, thus permitting induction of LTD and imparting a filter characteristic to the hippocampal circuit. It is not known to what extent these characteristics reflect modulation of the fast somatic or the slow dendritic GABA_A components, nor whether these two components are modulated similarly. In the present study we have characterized use-dependent and GABA_A-mediated depression of the two components of GABA_A-mediated inhibition.

Monosynaptic IPSCs were recorded from adult rat hippocampal CA1 cells in the presence of APV (40 μM) and CNQX (20 μM). Sharp electrodes filled with CaAcetate (3M) or CaCl_2 (3M) and 4X-114 were used to record GABA_A fast and GABA_A slow in response to electrical stimulation of stratum pyramidale and stratum lacunosum-moleculare. As described previously (Neuron 10:189-200, 1993) it was found that paired-pulse depression of GABA_A slow follows the time course described previously for stratum radiatum responses, with a slow onset and maximal depression of 60% at approximately 160 ms. In contrast, GABA_A fast was depressed maximally by 40% at 5 ms, and declined at later time points, so that only 10% depression remained at 160 ms. This suggests that GABA_A fast was also relatively resistant to the action of CaAcetate, being maximally depressed by only 50% at 10-20ms, with no effect at 1 μM, whereas GABA_A slow was depressed by 50% at 0.3 μM and completely eliminated at 1 μM. CDP 15348 only partially reversed the paired-pulse depression of GABA_Afast but eliminated paired-pulse depression of GABA_A slow.

These experiments demonstrate significant differences between the presynaptic characteristics of the two components of GABA_A inhibition, suggesting that different mechanisms may underlie use-dependent depression, and further supporting distinct functional roles for the two systems.

**CHARACTERIZATION OF AN ATRP RECEPTOR ON A CHOLINERGIC PRESYNAPTIC NERVE TERMINAL. X.P. Sun and J.F. Stanley. Synaptic Mechanisms Section, NINDS, Bldg 36, Rm 5A27, NIH Bethesda, MD 20892.**

ATP activates a cation channel on the presynaptic nerve terminal of the cholinergic calyx-type synapse in chick ciliary ganglion (Sci. N.S. Abst. 1991). We have examined the pharmacology of this receptor using purinergic agonists and antagonists. Calyx nerve terminals and their postsynaptic ciliary neurons were isolated and patch clamped (J Neurosci, 11:985). ATP induced fast-activating currents in the nerve terminal and neuron with reversal potentials of about 0 mV. The nerve terminal ATP current inactivated slowly whereas the neuron exhibited both fast and slow inactivating current components. The P_2 agonist, ATPs and 2MeS-ATP mimicked ATP while the P_2a and P_2a agonists, adenosine and β,γ-methylene ATP were ineffective. The P_2 blockers, reactive blue and suramin, were ineffective on the nerve terminal and eliminated only the fast-inactivating current.

Our results suggest that the slowly inactivating ATP conductance on this presynaptic nerve terminal is mediated by neither P_2a nor P_2b-type receptors but by an as yet unidentified receptor type. Since ATP is stored with ACh in secretory vesicles, this presynaptic ATP receptor may play a role in the feed-back modulation of transmitter release.

**RECEPTOR-EFFECTOR COUPLING MECHANISM UNDERLYING THE DESENSITIZING PRESYNAPTIC GABA_A RECEPTOR-MEDIATED INHIBITION OF EXCITATORY TRANSYNAPTIC SYNAPSES. J.L. Plaut.**

We previously proposed the existence of a desensitizing presynaptic GABA receptor (DS-GABA) mechanism as a functional unit in a system in which the postsynaptic membrane only expresses N-methyl-D-aspartate (NMDA) receptors (Plaut et al., Soc. Neurosci. Abstr. 18:792). In the present study, whole-cell patch-clamp recordings from hippocampal neurons in CA1 revealed two distinct synaptic components, an excitatory component driven by DS-GABA receptor and the non-desensitizing (NDS-GABA) receptor. Both receptors display distinct excitatory transynaptic synapses which express postynaptic NMDA and non-NMDA receptors. Superfusion of Ba^2+ (1mM) failed to block baclofen-induced activation of DS-GABA or NDS-GABA inhibition of spontaneous NMDA or non-NMDA receptor-mediated synaptic currents, respectively, indicating that neither receptor is coupled to a K^+ channel. Pretreatment of cell cultures with pertussis toxin (1.0 μg/ml, 24-48 hr) selectively blocked DS-GABA but not NDS-GABA inhibition in some cultures suggesting receptor coupling to separate G-proteins. Pretreatment and co-superfusion of pertussis toxin (1.0 μM) blocked both DS-GABA and NDS-GABA receptor-mediated inhibition. Pretreatment with dicyclohexylcarbodiimide 1 μM) blocked NDS-GABA inhibition, whereas DS-GABA inhibition remained blocked. These results suggest that preyective DS-GABA and NDS-GABA receptors may regulate the synaptic release of glutamate through distinct receptor-effector coupling mechanisms. This raises the possibility for selective modulation of transmission at functionally distinct glutamatergic synapses.

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**DOES MUSCARINE ACTIVATE K^+ CHANNELS AT PRESYNAPTIC TERMINALS IN HIPPOCAMPAL AREA CA1. M.G. Rapistain* & S. Wachtler and W.F. Coinger. Dept of Pharmacology, Univ. of Alberta, Edmonton, Alberta, Canada, T6G 2H7.**

Prejunctional inhibition of glutamate release from hippocampal area CA1 can be activated by the activation of several different receptors, including GABA_A, Adenosine_A, Neuropeptide_Y, and a muscarinic acetylcholine receptor. The mechanism whereby these receptors act at presynaptic terminals cannot be recorded directly. Nevertheless, if they all act via an identical mechanism, their actions should be equally affected by manipulations that affect transmitter release. 4-Aminopyridine (30 μM) reduces inhibition mediated by the Y, and GABA_A receptors, but not that caused by muscarine. We found that NMDA (1 mM) reduced the inhibition caused by muscarine. Here, we tested the hypothesis that muscarinic receptors activate presynaptic K^+ channels, by examining its actions when either the blocking Ba^2+ is removed.

Extracellular recordings were made of population EPSP's evoked area CA1 by stimulation of stratum radiatum with 500 μA. We examined the effect of 200 μM muscarine on the EPSP recorded from the stratum radiatum with the same stimulation, and 0.36 mM Ba^2+ reduced the effects of Ba^2+ on the EPSP. These observations are not consistent with muscarinic receptors inhibiting glutamate release by the same mechanisms as ACh or GABA_A receptors, respectively. The observed response is consistent with the activation of muscarine on a presynaptic K^+ channel. If this were the case, the block of presynaptic K^+ current by Ba^2+ would reduce the resistance of the terminal, and activation of another K^+ channel by muscarine would bring the membrane potential closer to E_K, thus further from threshold than under control conditions.

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**NICOTINE FACILITATES AND DEPRESSES GLUTAMATERGIC SYNAPTIC TRANSMISSION AT A CNS SYNAPSE IN VITRO. D.S. Mckeeber* & L.W. Role. Dept of Anatomy & Cell Biology in the Center for Neurobiology and Behavior, Columbia University, 722 W 168 St, NY, NY 10032.**

Although habenular neurons from both chick and rat express nicotinic ACh receptors (nAChRs). Physiological studies demonstrate that these receptors are not involved in synaptic transmission. Autoradiographic studies localise many CNS nAChRs to terminal fields, including that of the habenula-IPN projection. We tested whether habenular nAChRs could play a role in modifying glutamatergic transmission in vitro.

Embryonic habenular cultures were cultured in dissociated IPN neurons, a principle target of habenular neurons in vivo. Whole cell recording from IPN neurons located near habenular explants revealed potentials of about 10 mV. Embryonic habenular explants revealed synaptic activity that is blocked by a mixture of the glutamatergic antagonists, CNQX and APV, and not by the nicotinic antagonist, mecamylamine, demonstrating glutamatergic synapses at these habenular-IPN synapses. Stimulation of the habenular neurons by an extracellular stimulating electrode evokes post synaptic currents (EPSCs). Application of a low concentration of nicotine (0.5 μM) increases the amplitude of EPSCs, returning to baseline within 30-60sec, in the continued presence of the nicotine.

This concentration regimen caused an increase in the frequency of spontaneous miniature IPSCs recorded in the presence of 4μM TTX, without affecting the amplitude of these currents. This supports the idea that the receptors responsible for nicotine-induced synaptic facilitation are located on the preysynaptic cell.

In contrast, application of nicotine at 10-20μM increases the percent failure of the evoked EPSCs, possibly due to shunting of theulking action potential. The mechanisms and nAChR subtypes responsible for these nAChR-mediated modifications in synaptic transmission are not yet known. Supported by a grant from the U.S. National Institutes of General Medical Sciences (Grants 4415610 to L.W.R and 5459395 to I.M.)
**462.11**

α-LATROTOXIN ENHANCES SYNAPTIC TRANSMISSION BUT DOES NOT PREVENT PRESYNAPTIC INHIBITION INDUCED BY ADENOSINE, BACLOFEN AND OPIOID PEPTIDES IN HIPPOCAMPUS, M. Capuano*, B.H. Gähwiler and S.M. Thompson. Research Institute, University of Zurich, CH-8029 Zurich Switzerland

The black widow spider venom component α-latrotoxin (α-LTX) binds to the α-LTX receptor that is associated with presynaptic proteins, synapticcaptoprin, syntaxin, and Ca**2+** channels. These proteins control the docking of synaptic vesicles at the active zones and regulate exocytosis. Spontaneous mEPSCs (in the presence of TTX and bicuculline) and mIPSCs (in the presence of TTX, CNQX, and AP5) were recorded by means of whole-cell patch clamp in CA1 pyramidal cells of rat hippocampal slice cultures. Focal application of α-LTX (< 1nM) produced a 10-100 fold increase in the frequency of both basal mEPSCs and mIPSCs which persisted for at least 1 h. The α-LTX-induced activity was characterized by intermittent high-frequency bursts, and was only slightly affected by 100 μM Cd**2+**. Furthermore, α-LTX (with or without Cd**2+**) did not occlude the reduction in the frequency of miniature currents induced by 50 μM adenosine, 10 μM baclofen, and by 1 μM of the μ-opioid agonist FK 33-824. Therefore, these agents inhibit transmitter release by a mechanism that does not involve the modulation of the proposed α-latrotoxin/synaptotagmin syntaxin/Ca**2+** channel complex.

**462.12**

ACETYLCHOLINE RELEASE EVOKED BY ACTION POTENTIALS IN THE ABSENCE OF CALCIUM ENTRY IN FROG MOTOR NERVE: ANTAGONISM BY ADENOSINE. M. Watanabe, E.M. Silinsky*, R.S. Redman, J.K. Hirsh, B. Ouy, J.M. Hunt, S. Alfred and R.C. MacDonald, Dept. of Physiol., Pharmacol. and Biolog. Chem., Northwestern U., Chicago, IL 60611 and Evans, U. (39±9% of stimulated EPSC) was observed when AcCh was applied in Ca**2+**/syntaxin/Ca**2+** release experiments. This indicated that a voltage-sensitive Ca binding protein promotes phasic acetylcholine (ACh) release and ii) that the action of adenosine might be impaired by the functioning of Ca binding proteins associated with the synaptosomal apparatus. We delivered Ca via Ca-containing lipid vesicles (Ca liposomes) to motor nerve terminals in frog cutaneous pectoris muscle under conditions in which Ca entry cannot occur. Neurally-evoked AcCh release (recorded as multiquantal end-plate potentials) was generated by Ca liposomes suspended in solutions with no added Ca and containing either a) Mg (1-3 mM), b) Mg (1-3 mM) + Co(1 mM) or c) Mg (1 mM) + EGTA (1 mM). Adenosine (10-100 μM) inhibited AcCh release generated by nerve impulses in the absence of Ca entry. Both electrophysiological and morphological studies using confocal laser microscopy suggest that the liposomes are interacting with the nerve terminal membranes and delivering their entrapped contents to the cytoplasmic milieu without leakage to the exterior of the cell. The results suggest that action potentials in vertebrate motor nerve endings can promote the synchronous, physiologically-functional form of AcCh release in the absence of Ca entry provided the cytoplasmic Ca is elevated by Ca liposomes. Such evoked AcCh release in the absence of Ca entry is antagonized by adenosine. (Supported by NIH Grants NS12782 and NS30795).

**462.13**

GENETIC EVIDENCE THAT MODULATION OF BOTH K AND CA CURRENTS CONTRIBUTE TO α2-ADRENERGIC-RECEPTOR-MEDIATED INHIBITION OF HORMONE SECRETION. P.P. Lakhiani, D.M. Levinger and L.E. Liberti*. Department of Endocrinology and Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232.

The α2-adrenoceptor (α2-AR), a member of the G-protein-coupled receptor family, inhibits neurotransmitter and hormone release in nearly every system. Point mutation of the highly conserved aspartate residue in the putative second transmembrane segment of the receptor to asparagine (D79N/α2-AR) results in loss of coupling of the receptor to G-protein (Gα2) without any profound effect on the receptor-mediated inhibition of adenylate cyclase (AC) and calcium (Ca) current (Science, 257:971, 1992). We have utilized this unique α2-AR as a tool to examine the precise involvement of these effector systems in the inhibitory action of the α2-AR. The effect of activation of stably transfected wild-type (D97 WT) and D79N α2-AR-expressing aortalorticortic hormone (ACTH) secretion from mouse anterior pituitary cells (A1720) was examined. The basal ACTH secretion was not significantly different in A1720 cells expressing D97 WT and D79N α2-AR. Treatment with the β-adrenoceptor agonist isoprenaline resulted in concentration-dependent stimulation of ACTH secretion which, again, was not significantly different in the two cell lines. However, activation of D79N α2-AR by the maximal concentration of the α2-AR agonist UK 14,304 resulted in significantly less inhibition of isoprenaline-stimulated ACTH secretion compared to that observed with D97 WT (395% vs 771% release). Next the possible involvement of AC in the α2-AR-mediated inhibition was examined. When ACTH secretion was stimulated by the cAMP analogue 8-bromo-cAMP, D97 WT and D79N α2-AR activation was still able to suppress the stimulated secretion. These results suggest that (i) only a part of the α2-AR-induced inhibition is mediated by cAMP current activation, and (ii) modulation of AC plays a minor role in the α2-AR-induced inhibition, implying that suppression of the remaining α2-AR inhibitory component. (Funded by HL 25182 and NS 30470)

**462.14**

PRESYNAPTIC ENHANCEMENT OF EXCITATORY SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS BY β-ADRENERGIC RECEPTOR ACTIVATION. P.J. Conn* and R.W. Greiss* IV. Department of Pharmacology and Program in Neuroscience. Emory Univ. Sch. of Med. Atlanta, GA 30322.

β-adrenergic receptor activation has effects in the hippocampus that might influence synaptic transmission as a result of activation of voltage-gated calcium channels and phosphorylation of synaptotagmin I. Direct stimulation of adenylyl cyclase with forskolin has been shown to potentiate transmission in area CA1, but it is not known if the β-adrenergic receptor-activated adenylate cyclase is similar effects. We now report that application of isoproterenol to hippocampal slices potentiated evoked excitatory postsynaptic currents (EPSCs) in CA1 pyramidal cells, and this response was potentiated in the presence of a phosphodiesterase inhibitor. Isoproterenol also resulted in the appearance of a late inward current that likely represents polyynaptic EPSCs. The potentiation of EPSC in response to isoproterenol was blocked by 889, an inhibitor of cAMP-dependent protein kinase. In addition, isoproterenol induced an increase in the frequency of spontaneous miniature EPSCs, but did not affect the amplitude of mEPSCs or currents elicited by direct application of AMPA. These results suggest that presynaptic β-adrenergic receptor mediated responses may act to enhance synaptic transmission in area CA1 via activation of cAMP-dependent protein kinase.

**462.15**

NOREADRENALINE PRESYNAPTICALLY INHIBITS THE EPSCS OF SYNAPTIC TRANSMISSION TO NEURONS. T. Miyazaki, H. Kobayashi, S. Mochida and T. Tosaka Dept. of Physiol., Tokyo Medical College, Tokyo 160, Japan

Excitatory postsynaptic currents in spinal cord thin slices from P7 to P12 rats were identified by DiI preinjection to the superior cervical ganglion. Under the whole-cell configuration of slice-patch method, we recorded a mono-synaptic EPSC in the SPMs evoked by stimulation at the nerve intercalate, containing strychnine (5μM) and bicuculline (10μM). The EPSCs were abolished by kynurenam (2μM) or CNQX (10μM) and were inhibited reversibly by the EPSCs in about 80% neurons tested. The inhibitory effect was dose-dependent, reaching maximal inhibition (50-100%) at a higher dose of NA (500μM), about 50 % of EPSC was remaining. NA did not induce any appreciable shift of the reversal potential of the EPSC which was around zero mV. Glutamate current generated by pressure application to the soma was not affected by NA, though the simultaneously recorded EPSC was not inhibited. NA effect completely disappeared in the photolamine (10μM) solution. The results suggest that noradrenergic nerves inhibit the release of glutamate through activation of the α1-adrenergic receptors.

**462.16**


Previous studies have shown that dopamine (DA) receptor agonists and antagonists may induce dopamine release and receptor effects. We have recently shown that endogenous DA. We used a model of intrastriatal microdialysis in freely moving rats to study the effect of pergolide, a D1 and D2 DA receptor agonist, on the biotransformation of endogenous and exogenous L-DOPA. Levels of L-DOPA, DA, DOPAC and HVA were measured by high-performance liquid chromatography. Pergolide (2 mg/kg, i.p.) caused a 47 % decrease in basal striatal extracellular (EC) levels of DOPAC and a 65 % decrease in basal striatal EC levels of HVA, 60 minutes and 120 minutes after injection, respectively. EC DA became undetectable 60 minutes after injection of pergolide. L-DOPA (100 mg/kg, i.p.) was detected in 5 minutes after pergolide, produced significant increase in EC levels of L-DOPA, DOPAC, HVA and DA in rats with and without perfusion of 104 M pergolide. The DOPAC peak value was lower and was reached 60 minutes later in the group with pergolide.

This study demonstrates inhibitory effects of pergolide on release and metabolism of endogenous DA and on biotransformation of exogenous L-DOPA. These effects, probably mediated by DA autoreceptors, might have important pharmacological implications.
463.1 Cloning and expression of the P2X receptor reveals a new class of ligand-gated ion channel
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A cDNA encoding an ion channel gated by extracellular ATP (P2X receptor) was isolated from the rat vas deferens by expression cloning in Xenopus oocytes. When expressed in oocytes or in the HEK-293 cell line, this clone produces a receptor activated by nM concentrations of ATP, which shows strong desensitization. It is non-selective for cations and highly permeable to Ca2+2. It exhibits a P2X-type pharmacology, being activated by 2-methyl-5-ATP > ATP > d3-methyl-ATP, and inhibited by suramin and pyridoxalphosphate-6-azophenyl-2,4-dimethyl-3-phenylhydantoic acid (PDAPS). In outside-out patches excised from oocytes expressing the P2X clone, single channels of about 11 pS are activated by ATP. The cDNA consists of 1837 bp with an open reading frame predicting a protein of 206 amino acids. The protein sequence bears no homology to other ligand-gated ion channels and appears to have only two hydrophobic segments long enough to span the membrane. This receptor with novel structure probably represents the first member of a new class of ligand-gated ionotropic receptors.

463.2 Zn2+ POTENTIATES STEADY-STATE ATP ACTIVATED CURRENTS IN RAT NODOSO GANGLION NEURONS. A WHOLE-CELL AND SINGLE-CHANNEL STUDY. Jerry M. Wright* and Chao-Li Li. LMCC, National Institute on Alcohol Abuse and Alcoholism, NIH, Bethesda, MD 20892
Zn2+ and ATP are normally present in serum at low levels and are released from some neurons during synaptic activity. In whole-cell recordings, 10 μM Zn2+ potentiated steady-state currents in rat nodosus ganglion neuron cells by 18% in 0.5 μM ATP and 120% in 2 μM ATP. Fluctuation analysis of whole-cell currents suggested that the mechanism was increased burst-duration. Single channel recordings indicated the presence of several channel conductances. There was a primary conductance of 25-35 pS plus other low conductances present in the baseline noise. 10 μM Zn2+ did not alter the conductance of the 25-35 pS ATP-activated channel but did increase the probability of opening. Kinetics of the 25-35 pS channel were complex with fast openings and closings in addition to substate transitions and bursting. The response of the low conductance channels to Zn2+ was highly variable, suggesting multiple types of low conductance ATP-activated channels.

463.3 BIPHASIC MODULATION OF THE GLYCINE RECEPTOR BY Zn2+. A.R. Blomesthal, E. Goldwater, D.R. Frritch* and N.L. Harrison*. Deps. of Anesthesia and Pharm./Phys. Sciences, Univ. of Chicago, Chicago, IL, and *Dep of Pharm, Univ. of Pennsylvania, Philadelphia, PA. Zn2+ exerts a subunit-dependent inhibitory action on the GABA receptor (Drugaul et al., Neuron, 5: 781-8). We have studied the effects of Zn2+ on the structurally related glycine receptor (GLY-R). In whole-cell patch clamp experiments on cultured rat spinal neurons, Zn2+ potentiated GLY-activated CI- currents at a concentration of 500μM, but blocked the current at a concentration of 50μM. The native GLY-R is an αβ heteropentamer (Langosch et al., PNAS, 85:7394-4). Further experiments were conducted by transient expression of receptor in HEK 293 cells by transfection with cDNAs encoding the α2 and β subunits of the human GLY-R. When the α2 subunit alone was expressed, GLY-activated CI- currents were blocked by strychnine (10μM) and inhibited by picotoxin (PTX) concentrations as low as 1μM, potentiated by Zn2+ concentrations between 0.2 and 2μM, and blocked by concentrations > 5μM. When the α2 and β subunits were expressed together, PTX sensitivity was abolished, as in spinal neurons. However, the addition of the β subunit had little effect on Zn2+ sensitivity. It seems likely that Zn2+ acts on the α subunit, while the β subunit has little effect on the Zn2+ interaction. The dual action of Zn2+ in modulating GLY-Rs suggests that two distinct binding sites may be present. The biphasic action of Zn2+ on the GLY-R is interesting in view of other reports of dual Zn2+ modulation of ligand-gated ion channels (Hollmann et al., Neuron, 1994-95). The possible conservation of Zn2+ modulatory sites may prove significant in the case of the NMDA receptor, which also carries a binding site for glycine as an essential co-agonist. We thank Heinrich Betz for the β subunit cDNA.

463.4 EFFECTS OF NICOTINIC ANTAGONISTS ON GLYCINE RECEPTORS. V.E. Wotring and K.W. Yoon. Department of Pharmacology and Physiological Science and Division of Neurosurgery, Saint Louis University Health Sciences Center, St. Louis, MO 63104.
Since certain nicotinic antagonists can inhibit GABA induced currents (Wotring and Yoon, Soc. Neurosci. Abs., 52:93), we pursued the possibility that these same agents might also inhibit glycine induced currents. Measurements of glycine evoked currents were made using the whole cell patch clamp technique with rat hippocampal cells grown in culture for 10 - 15 days; drug applications were made with a U-tube. In these experiments, GABA currents and glycine currents could be distinguished using the w/cusculine and strychnine. Strychnine (1μM) blocked 63% (p<0.001, n=4) of the control glycine (100μM) current while it did not alter a 30 μM GABA current (p=0.02, n=4). Correspondingly, baclofen (1μM) decreased the current but did not affect the glycine current. Application of the nicotinic antagonist, d-tubocurarine at a concentration of 100 μM reduced glycine currents by 37% (p<0.001, n=5). 100 μM trimethaphan camylate diminished glycine currents by 63% (p<0.001, n=8). Nicotinic antagonists that we have previously found to not significantly affect GABA currents had unexpected effects on glycine currents. 100 μM d-tubocurarine blocked 51% (p<0.001, n=5) of the glycine current. Application of 1 μM mecamylamine resulted in a nearly complete blockade (89%, p<0.001, n=6) of glycine current. However, 1 μM hexamethonium did not alter the control glycine current (p>0.2, n=6).
The ability of these nicotinic antagonists to block glycine and GABA currents forebodes structural similarity among these ligand-gated channels. However, the differential effect of mecamylamine on GABA and glycine currents suggests common variances in the mechanisms of antagonism.

Four proteins isolated from rat brain synaptic membrane have molecular weights of 67-70, 53-58, 41-46, and 31-36 kDa and contain binding sites for NMDA, glutamate, 2-AAP, TCP, and glycine (Kamin et al., Nature, 354, 70, 1991). A 40-42 kDa glycine-binding protein has been isolated by affinity chromatography on 7,5-dichlorokynurenamine (7,5-DCKA) column (Babcock et al., Neurosci. Abs., 1994). Polyclonal antibodies raised against the 40-42 kDa proteins purified from 41-43 kDa protein in synaptic membranes. These antibodies were used to screen a rat hippocampus cDNA library in ZAP vector and an ~1.9 kbp clone expressing the antigenic protein was identified. The open reading frame of this clone describes a unique protein of 470 amino acids (52.8 kDa). Northern blot analysis of brain RNA revealed hybridization of the cDNA probes to transcripts of ~2.0 kb. RNA levels were expressed approximately equal in hippocampus, cerebral cortex, and cerebellum. Lane 1 corresponded with the pIII vector carrying the 1.9 kbp insert expressed a fusion protein that was recognized by the anti-glycine binding protein antibodies. The fusion protein was purified by IPTG induced E. coli extract using 5, 7-DCKA-derived column. Two defined proteins identified two binding sites for [3H]glycine with estimated K2 of 124 nM and 8.1 μM. The binding of glycine was displaceable by D-serine, 7,5-DCKA and HA-966. These results suggest that there are two classes of protein that may belong to a different subset of brain NMDA receptors (Supported by grants AA 04732 and DA003-91-G-0167 and an unrestricted grant from Parkinson's Disease).

An 40-42 kDa protein that has binding sites for glycine, D-serine, HA-966, and 7,5-dichlorokynurenamine was purified from rat brain synaptic membranes (Babcock et al., Neuroscience Abstr., 20, 1994). Polyclonal antibodies were developed against proteins of 41-43 kDa. These antibodies reacted specifically with an 60 kDa protein in synaptic membranes (Western blot analyses). Immunohistochemical studies in rat brain were performed using the antigen to the 41-43 kDa proteins. The results of these studies indicate that in detergent-permeabilized brain sections, the pyramidal neurons of the cerebral cortex and the hippocampus exhibit strong immunoreactivity with the anti-glycine-binding protein antiserum. Most heavily labeled were the pyramidal neurons, including their proximal dendrites, in the CA2 region of the hippocampus. The granule cells of the cerebellum and the dentate gyrus were also strongly labeled. A comparison between the labelling observed with the antiserum to the 63-70 kDa glutamate-binding protein, the 53-58 KDa CPP-binding protein, and the glycine binding protein indicates that all three antibodies label some of the same populations of neurons with only small differences observed in the pattern of labelling. The antibodies raised against these three proteins may be useful probes for the determination of the distribution of a subpopulation of glutamate and NMDA-like receptors in brain. (Supported by grants AA 04732, DA003-91-G-0167 and HD 05258.)

A group of synaptic membrane proteins with sizes of 67-70, 53-58, 41-46, and 31-36 kDa has recognition sites for NMDA receptor ligands, such as a glutamate receptor subtype, a Ca²⁺ channel, and a GABA receptor. It is possible that these proteins are breakdown products of the NMDAR1/G2 proteins or that they are distinct entities. An ~40 kDa protein that has binding sites for glycine was purified from a rat synaptic membrane preparation. Affinity chromatography first through a matrix derivatized with 5,7-dichlorokynurenam (5,7-DCKA), then through a derivatized with O-OH-quinolone. An 40-42 kDa protein was isolated and this protein bound [3H]glycine in a symmetric-symmetric-intensive manner and with a stoichiometry of 1 mol glycine/mol protein. The glycine binding sites of the protein had a low affinity (3-8 μM) for glycine. D-, Serine, 5,7-DCKA, and HA-966 inhibited glycine binding to the protein. Polyclonal antibodies developed against proteins of 41-43 kDa electroeluted from SDS-polyacrylamide, which were recognized with an 60 kDa protein in synaptic membranes (Western blots). Treatment of solubilized synaptic membrane proteins with these antibodies led to the immunoreaction of approximately 60% of the [3H]Gly 2863-binding proteins. These observations indicate that a synaptic membrane protein of ~60 kDa size may be a symmetric-symmetric-intensive glycine receptor and that this protein is a component of a form of NMDA receptors. [Supported by grants AA 04732, DA00319-01A6, and a grant from Parke-Davis].

643.9 AGONIST-INDUCED CURRENTS IN IDENTIFIED NEURONS OF THE RAT DORSAL HORN. H. U. Zelhafer, T. Liebel, K. Brunner* and D. Swindulla, Institute of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nuremberg, D-91054 Erlangen, Germany.

Neurons within the dorsal horn of the spinal cord play a critical role in sensory information processing. In the present study, we have investigated ionic currents elicited by external application of the excitatory aminoacid neurotransmitter glutamate and of the noncompetitive neurotransmitters glycine and GABA. 250 μM thick transverse slices were cut from the spinal cord of 7 to 14 day old rats. Agonist currents were recorded from visually identified neurons in the dorsal horn of the rat spinal cord (lamin III to V) using the tight seal whole cell configuration of the patch clamp technique. Currents elicited by application of 100 μM L-glutamate showed a strong outward rectification in the presence of 1 mM Mg²⁺, indicating that a large portion of the current was through NMDA receptor channels. Currents induced by 100 μM glycine showed a transient and a maintained component. Both components reversed at the chloridie equilibrium potential. Neither run down nor wash out of the current could be observed with recordings that lasted up to 90 minutes. This indicates that the current was through chloride channels that are directly gated by glycine. GABA (10 μM) at a holding potential of -80 mV elicited inward currents which showed only a steady state component. Currents elicited by GABA displayed a rapid run down within 10 minutes of whole cell recording and were blocked by the GABReceptor antagonist CGP 36348 (200 μM) These findings indicate that GABA induced currents depends on the activation of GABAB receptors and on a rapidly diffusible intracellular molecule. Supported in part by a grant from the Hildgck Doerenkamp-Gerhard Zbinden Foundation to HUZ and DS.


The GABA receptor is a pentameric protein composed of various combinations of α, β, and γ subunits. At least one α, one β, and one γ are necessary for a fully functional GABA receptor expressing α (benzodiazepine) pharmacology. In the present report we describe the development of a recombinant cell line expressing a βγ α3 subunit of the GABA receptor. Following transfection and selection in G418 several clonal cell lines were isolated and tested for [3H]-muscimol binding and [3H]-flumazenil binding activity. Among the cell lines one exhibited 200 fmol of [3H]-flumazenil binding sites per mg membrane protein and was selected for further characterization. Equilibrium saturation analysis revealed a Kᵰ value of 0.39 nM for [3H]-flumazenil in good agreement with the Kᵰ values of 1.4 μM for [3H]-flumazenil using transiently-transfected α3βγ GABA receptor constructs and rat cerebellar membrane preparations. The Kᵰ values of diapace, CL 218872 and zolpidem to inhibit [3H]-flumazenil binding to this cell-line were 18 nM, 210 nM and 40 nM, respectively. The concentration-dependency of GABA-induced chloride current in this cell-line gave an EC₅₀ value of 6.3 μM as determined by the whole cell patch-clamp technique. GABA (1 μM)-induced chloride current was poteniated by diazepam and zolpidem with EC₅₀ values of 36 and 160 nM, respectively. In conclusion, these properties indicate that a functional GABA receptor subtype with α3 pharmacology has been stably expressed in this transfected cell-line.


The symmetrical compound bis (2,6,6-tetramethyl-4-piperidinyl) sebacate (BTMPS) is a use-dependent inhibitor of nicotinic AChR. The active portions of BTMPS are the tetramethylpiperidine (TMP) rings. However, inhibition of neuronal nAChR by TMP is much more rapidly reversible than inhibition by BTMPS, suggesting prolonged inhibition of neuronal nAChR may result from binding of the conjugated compound to multiple sites within the receptor. A compound related to BTMPS, but differing by four carbons in the piperidine rings, showed a dramatic loss of inhibitory activity.

The inhibition of neuronal nAChR by BTMPS is rapidly reversible unless a neuronal beta subunit is substituted for the muscle beta subunit. This suggests that normal muscle-type receptors may contain a single site for BTMPS inhibition and that the expression of a neuronal beta subunit provides an additional site. The sequences of the neuronal beta subunits differ from the muscle beta subunits in regions that are extracellular vestibule forming domains. The muscle delta subunits are similar in sequence to the neuronal beta subunits in these regions, while the muscle gamma subunit more closely resembles the muscle alpha subunits. Over-expression of the delta subunit (omitting gamma subunit RNA) has the same effect on the reversibility of BTMPS inhibition as a neuronal beta subunit for the muscle beta subunit. Compared with muscle-type and delta-less, recombinant C. elegans a subunit, the neuronal gamma subunit is sensitive to mecamylamine (p<0.01), 2 showed a significant increase in divalent ion permeability (p<0.05), and 3 had greater inward rectification (measured by chord conductance). Our data suggest that the domains which control noncompetitive inhibition may also regulate ion permeation properties.

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Cholinergic nicotinic receptor desensitization was studied in cultured rat myoblasts using the whole cell configuration of the patch clamp technique. Whole cell currents were recorded following treatment of desensitized applications (40s-2min) of ACh. The current reached a peak in sec and declined to a steady state level during agonist application. The rate of onset of desensitization was voltage dependent and accelerated by an increase in extracellular [Ca\textsuperscript{2+}]. Simultaneous measurement of [Ca\textsuperscript{2+}]\textsuperscript{i}, using dual excitation microspectrofluorometry with fura-2 loaded myoblasts indicated a significant permeability of activated ACh receptor channels to calcium. Agonist-induced currents as well as increases in [Ca\textsuperscript{2+}]\textsuperscript{i}, were blocked by prior exposure of myoblasts to α-bungarotoxin. Acceleration of the onset of desensitization by calcium required external calcium during receptor activation. The [Ca\textsuperscript{2+}]\textsuperscript{i} reached a peak following ACh application and declined slowly to basal levels. An increase in [Ca\textsuperscript{2+}]\textsuperscript{i} produced by photolysis of cytosolic DMS-Nitrophen-Ca\textsuperscript{2+} complexes also accelerated desensitization onset in the absence of external calcium. These results suggest that Ca\textsuperscript{2+} entry through ACh-activated channels is an important pathway for the regulation of the ACh receptor-channel by providing Ca\textsuperscript{2+} required for intracellular modulatory mechanisms. The technique of photolesere calcium will be used to determine the minimum amplitude and duration parameters of the [Ca\textsuperscript{2+}]\textsuperscript{i}, required for the modulation of the ACh receptor and desensitization kinetics (Supported by MDA).

ASTROGLIAL AND NEURONAL ADRENERGIC RECEPTORS LINKED TO CHANGES IN [K\textsuperscript{+}] AND [Ca\textsuperscript{2+}]\textsuperscript{i}: EXHIBIT DIFFERENT CHARACTERISTICS. M. Nilsson, T. M. Marderman, P. S. Eriksson, L. Hansson and L. R. Petrussevs, 1, 2, 3.

Institute of Neurobiology 1, Departments of Neurology 2, University of Gottingen and Department of Cell Biology, Faculty of Health Sciences, University of Linköping, Sweden.

Various adrenergic agonists were investigated for their effects on intracellular concentrations of calcium ([Ca\textsuperscript{2+}]\textsuperscript{i}) and potassium ([K\textsuperscript{+}]\textsuperscript{i}). The calcium sensitive dye fura-2 and the potassium sensitive dye PBF were used in mixed astroglial/neuronal primary cultures from newborn rat cerebral cortex.

We found considerable differences in the adrenergic-evoked changes in [Ca\textsuperscript{2+}]\textsuperscript{i} and [K\textsuperscript{+}]\textsuperscript{i} between the two celltypes. Norepinephalin (NA), phenylephrin (phe; α1-agonists), clonidine (clon; α2-agonist) and isoproterenol (iso; β-agonists) were all able to increase calcium and decrease potassium concentrations with one exception; clonidine could not induce potassium responses. In neurons, NA and phe evoked calcium transients while clon and iso did not. NA and clon were able to elicit potassium reductions but no responses were seen with phe and iso stimulation. The astrocytes responded in general with up to 4 different types of calcium response patterns while the neurons only exhibited 2 types. After adrenergic stimulation, no oscillations in intracellular calcium were seen in the neurons, but could on the other hand, in the astrocytes, the agonist-induced reductions in [K\textsuperscript{+}]\textsuperscript{i} always persisted at the lower level in the presence of the agonists. The results point out the astroglial cellpopulation as potentially important receivers of adrenergic input. The results also show that astroglial and neuronal changes in [Ca\textsuperscript{2+}]\textsuperscript{i} and [K\textsuperscript{+}]\textsuperscript{i} exhibit different characteristics which might be of great importance for the understanding of the basic principles involved in the homeostasis of extracellular calcium and potassium concentrations.
LIGAND-GATED ION CHANNELS I

663.19 POINT MUTANTS OF THE INHIBITORY GLYCINE RECEPTOR α1 SUBUNIT ASSOCIATED WITH HEREDITARY HYPEREKPLEXIA DISPLAY REDUCED AGONIST AFFINITY AND EFFECT IN SINGLE CHANNEL CONDUCTANCE.
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Native adult inhibitory glycine receptor (GlyR) is a pentameric ligand-gated ion channel protein composed of two different subunits (α1 and β) in a stoichiometry of 2:2. Hyperkplexia (STHE) is a human autosomal dominant neurological disorder associated with point mutations in the gene encoding the α1 subunit of the GlyCl receptor (Ann. Neurol. 35:135-138, 1994). According to an arginine residue bordering the channel forming transmembrane region M2 at position 271 is substituted to either tyrosine or glutamine.

We have determined the functional properties of these GlyR mutants on heterologous expression in Xenopus oocytes and kidney 293 cells. When analysing homomeric channels, glycine concentrations eliciting half-maximal responses (EC50) were approximately 100-fold increased in comparison with wild-type α1 GlyR. Further, we observed drastic decreases in maximal whole-cell currents (up to 90%) and a reduction in single channel main conductances. In addition, the glycine agonists β-alanine and taunine did not elicit detectable currents but no significant change in affinity for the competitive antagonist strychnine could be observed. Coexpression of the mutants with wild-type α1 subunits in different ratios resulted in mixed channels with intermediate glycine affinities and maximal currents depending on the ratio of the expressed subunits. Interestingly, coexpression of the mutants with both α1 and β diminished the effects of the mutations in comparison to coexpression with α1 alone.

Our results imply that the phenotype of STHE patients is the consequence of diminished agonist affinity and efficacy of the mutant GlyRs.

663.20 ISOLATION AND CHARACTERISATION OF THE MOUSE GLYCINE RECEPTOR β SUBUNIT GENE M.Fischer, H.Betz* and J.Kuhse. Max-Planck-Institute for Brain Research, D-60021 Frankfurt (Main), Germany.

The structure of the mouse glycine receptor β subunit gene was deduced from overlapping genomic lambda clones. Subcloning and sequencing of the identified ten exons which are spread over a distance of more than 50 kb. Comparison of the β gene with the previously characterized glycine receptor α subunit genes revealed high structural homology. However, there are some notable differences. Whereas the 5' non-coding region of the α gene is encoded by the first exon, the respective region of the β gene is separated by one intron. Also, the transmembrane region 2 is encoded by two exons, a finding similar to the GABA<sub>B</sub> receptor β gene structure.

The different glycine receptor subunit genes exhibit developmental and spatial specific expression in spinal cord. These expression patterns should reflect complex regulation at the level of transcription. Therefore, we are interested in a detailed characterization of the promoter and upstream regions of the glycine receptor β gene. Trancription start points were mapped 1.5 kb upstream of the start codon. To identify regulatory elements, transient expression studies with reporter gene constructs in neuroblastoma and control cell lines are being pursued.

LIGAND-GATED ION CHANNELS: ALCOHOL MODULATION

664.1 STUDY OF ETHANOL EFFECTS ON TM II DOMAIN MUTANTS OF THE NMDA RECEPTOR CHANNEL.
K. Masood*, C. Wu and F. F. White, Lab. Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892.

The N-methyl-D-aspartate (NMDA) receptor is one of the key neurotransmitter-gated ion channels, as it is involved in important physiological and pathophysiological aspects of nervous system function. NMDA receptors have been found to be affected by psychoactive drugs such as ketamine, phencyclidine and ethanol, possibly contributing to the behavioral effects of these drugs. Earlier we reported differential ethanol sensitivity of NMDA receptor subunit combinations (Mol. Pharmacol. 49:524, 1996). However, the mechanism by which ethanol exerts its effect on NMDA receptors is unknown. The transmembrane II (TM II) domain of NMDA receptor subunits is thought to constitute the pore of the channel. Single amino acid mutants in and around the TM II domain of NR1 were coexpressed with NR2A in Xenopus oocytes and the effect of ethanol on NMDA activated ion current was tested using the two-electrode voltage-clamp method. The NMDA activated-current of the native subunit combination was inhibited 41% by 100 mM ethanol. The ethanol inhibition for charged mutants was 43% for E598Q and 42% for D599N. The inhibition for polar mutants was 39% for T602G and 38% for S605G. The observations suggest that these charged and polar amino acid mutations did not significantly change ethanol inhibition of NMDA-activated current flow through the mutated channels.

664.2 ETHANOL SENSITIVITY OF RECOMBINANT GLUTAMATE RECEPTOR EXPRESSED IN XENOPUS OCYTES IS SIMILAR TO THAT OF NEURONS.
B.E. Akinbola* and F.F. White. Lab. of Molecular and Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892.

The Ethanol(ETOH) sensitive neuronal AMPA(α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) type glutamate receptors(GluR), have multiple subunits designated as GluR1-4(Cai, Science,249:556-560,1990), which exist in two distinct "splice variant" versions known as the FLIP and FLOP (Science,249:1580-1585,1990). We have examined the ETOH sensitivity of recombinant GluR1-3 FLOP receptor combinations expressed in Xenopus oocytes, using the two electrode voltage clamp technique. ETOH concentrations of 10-500 mM caused 50% inhibition of currents activated by 200mM kainate. The GluR1+2+3 heteromeric combination current exhibited the highest sensitivity to ETOH, with an IC50 value of 176mM. The response of GluR3 homomer receptor subunit to ETOH had an IC50 value of 212mM. The sensitivity of GluR3 and GluR1+2+3 subunits to inhibition by ETOH is similar to the reported sensitivity of non-NMDA glutamate-activated current in hippocampal neurons(Ann. Med.,22:247-252,1990). By contrast, the FLIP forms of GluR expressed in human embryonic kidney (HEK) 293 cells are reported to be more sensitive to ETOH(Neurosci. Letters,159:83-87,1993) than non NMDA glutamate-activated current in hippocampal neurons or the FLOP forms of GluR in our experiments.

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The neurosteroid pregnenolone can modulate the activity of GABAA, glycine and NMDA receptors. Ethanol inhibits NMDA-activated current but whether inhibition is affected by the modulatory actions of neurosteroids is not known. We investigated the effect of pregnenolone on ethanol inhibition of NMDA-activated current using the mouse NMDA receptor subunits ε1/ε2/ε3/ε7/ C-ε2/2 expressed in Xenopus oocytes. Membrane current was recorded using the two-electrode voltage-clamp technique at a holding potential of -70 mV. Ethanol inhibited the current concentration-dependently driven by 10 mM Ca2+ (in the presence of 10 mM MPA) in a concentration-dependent manner over the range 10 to 250 mM; the EC50 was 243 mM. Ethanol, 100 mM, decreased the Emax of the NMDA-activated current, but did not affect the EC50 of Hill coefficient. Pregnenolone sulfate potentiated NMDA-activated current in a concentration-dependent manner over the concentration range 0.3-100 μM. The EC50 of the concentration-response curve was 4.9 μM and the slope was 1.2; these values were not significantly changed by the presence of 10 mM ethanol. The potentiating effect of NMDA-activated current by pregnenolone did not affect the percentage inhibition by 100 mM ethanol of NMDA-activated current. The results suggest that the modulatory action of pregnenolone does not affect the mechanism involved in ethanol inhibition of NMDA receptor subunits ε1/ε2/ε3/ε7/ C-ε2/2.

CHRONIC ETHANOL TREATMENT UPRREGULATES NMDA RECEPTOR FUNCTION AND BINDING IN MAMMALIAN CORTICAL NEURONS. X.-L. Hu1,2, M.A. Jayorsa,1,2 and M.K. Ticku1,2. Dept of Pharmacology1 and Psychiatry2, The University of Texas Health Science Center, San Antonio, TX 78284-

In the present study we investigated the effects of chronic ethanol exposure on NMDA receptor-mediated increase in intracellular Ca2+ concentration ([Ca2+]i) and [3H]MK-801 binding in cortical neurons. NMDA increased the (Ca2+)i in a dose-dependent manner with an EC50 of 12 μM. Chronic exposure to the cortical neurons to ethanol (50 mM, 5 days) did not produce any changes in the cell protein, morphological appearance and the resting [Ca2+]i; however, it significantly enhanced the NMDA-mediated increase in [Ca2+]i. The EC50 value of NMDA was not significantly altered following chronic ethanol exposure, but its Emax value was increased by ~40%. This enhancement of NMDA-receptor response following chronic ethanol treatment was reversed by concomitant chronic exposure of the cortical neurons to the NMDA competitive (20 μM CPP) and non-competitive (1 μM MK-801) antagonists, but by not the non-NMDA receptor antagonist CNQX (10 μM) and the L-type calcium channel blocker, nitrindipine (10 μM). In chronic ethanol exposure increased the specific [3H]MK-801 binding in cortical neuronal membrane by ~20%. Taken together, these results suggest that chronic ethanol exposure upregulated the NMDA receptor function in cortical cultured neurons, and this increased NMDA receptor function is a NMDA receptor mediated process. The altered NMDA receptor function may be responsible for the ethanol-induced tolerance and withdrawal syndrome.

DIFFERENTIAL SENSITIVITY OF NMDA- AND GABA-ACTIVATED ION CHANNELS TO ALCOHOLS OF VARYING HYDROPHOBICITY. Robert W. Poogie and Forrest F. Weight, Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892.

Alcohols have been shown to inhibit responses mediated by the N-methyl-D-aspartate (NMDA) receptor (NMDARP-71) (a glutamate binding protein). The mRNAs for both NMDARP-71 and NMDA-71 did change following three weeks chronic ethanol treatment. (Supported by AA00609, AA00115, and T32DA07501).


The effects of EtOH on whole-cell currents elicited by NMDA application of 50 (μM) plus glutamate (10 (μM)) were studied in neurons of the rat. NMDA-elicited whole-cell currents had distinct fast and slow decay components (τSP 263-859, 1992). When applied together with agonists via the NMDA (EtOH) (174 μl/min) caused a decrease in peak amplitude and in the amplitudes of the fast and slow components of the NMDA response. However, the inhibition of the NMDA response was not a monotonous function of EtOH concentration, which suggests the participation of a second receptor or inhibitory one. This irregular pattern was evident in concentration-response curves for individual neurons and for averaged data as well, indicating that this phenomenon was not an artifact of averaging. Additionally, an amplitude enhancement (rebound) was often evident after washing out EtOH (e.g., 2 min after washing out EtOH (522 μM), 4 out of 6 neurons showed a rebound). These results suggest a dual effect of EtOH, the potentiation being counteracted to different degrees by inhibitory effects, and are qualitatively similar to those reported earlier for single channels (BERS Lett. 247:6, 1989). A later onset potentiation by EtOH of the NMDA response was also observed. When EtOH was continuously present in the bath and was simultaneously applied to the cells via the U-tube together with agonist, there was a decrease followed by a progressive enhancement of the NMDA peak current. These results suggest that in addition to the inhibitory effects of EtOH on NMDA responses, two types of potentiation, one rapidly and the other of later onset, can also be observed. (Supported by USPHS Grant NS25296).

EFFECTS OF CHRONIC ETHANOL TREATMENT ON GLUTAMATE RECEPtor BINDING AND mRNA LEVELS IN THE ADULT RAT BRAIN. J.G. Rodolfa1, M.C. Rill1, B.E. Hugnes2,2, D.W. Walkerg, E.K. McClish3, and P.J. Cees4. Departments of Pharmacology and Neuroscience. University of Florida College of Medicine, Gainesville, FL 32610 and Departments of Pharmacology and Toxicology, University of Kansas, Lawrence, Kansas 66047.

The amino acid L-glutamate is a major excitatory neurotransmitter that is involved in many CNS functions including memory, LTP, and synaptic plasticity. To determine if chronic ethanol altered glutamate receptor binding, male Sprague Dawley rats were fed a liquid diet for three weeks with 36% calories coming from either sucrose or ethanol. Binding studies were performed on 6 μl brain sections using the NMDA specific ligands [3H]MK-801 and [3H]-CGP 96359 as well as [3H]-kainate and [3H]-AMPa. Autoradiography of the brain sections were quantitated using NIH image software. Ethanol significantly increased [3H]MK-801 binding (p<0.05) in many brain regions including the lateral geniculate nucleus (113%), stratum oriens of hippocampal CA1 (114%), and CA3 (112%). CGP binding was significantly increased (p<0.025) in the amygdala (110%), stratum oriens (125%) and stratum radiatum (121%) of hippocampal CA1, CA3 (126%), CA4 (123%), and dentate gyrus (117%). No significant changes were seen with either of the non-NMDA receptor specific ligands, AMPA or kainate. To determine if changes in mRNA levels corresponded to the increase in NMDA binding, total RNA was isolated from the cerebral cortex, hippocampus, cerebellum, and striatum. Northern analysis were performed using probes specific for both the NMDA1 receptor subunit and NMDARP-71 (a glutamate binding protein). The mRNA levels for both NMDA1 and NMDARP-71 did not change following three weeks chronic ethanol treatment. (Supported by AA00609, AA00115, and T32DA07501).
ALCOHOL INHIBITION OF ATP-ACTIVATED CURRENT IS NOT MEDIATED BY ACTIONS ON MEMBRANE LIPIDS OR INTRACELLULAR PROTEINS. Chavan 1st, Robert W. Peoples and Forrest F. Weight. Lab of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse & Alcoholism, NIH, Bethesda, MD 20892.

We have previously demonstrated, using whole-cell patch-clamp recording techniques, that isolated bullfrog dorsal root ganglion neurons, that for alcohols with a molecular volume equal to or less than 42.2 ml/mol, potency for inhibiting ATP-activated current was correlated with lipophilicity (order of effectiveness: 1-propanol > trifluoroethanol > monochloroethanol > ethanol > methanol). However, despite increased lipophilicity, alcohols with a molecular volume equal to or greater than 46.1 ml/mol (1-butanol, 1-pentanol, trichloroethanol and dichloroethanol) were without effect on the ATP-activated current. In the present study we found that the amplitude of the current activated by 2.5 μM ATP was decreased by the extracellular application of 100 mM ethanol by 45.2±3% (n=8 cells), and the inhibition was not significantly altered by intracellular application of 100 mM ethanol in the patch pipette (n=7; P>0.05). Inclusion of GTP-S (50 μM) or GDP-S (100 μM) in the pipette solution also failed to reverse the inhibitory effect of ethanol. These results suggest that alcohol inhibits the function of this neurotransmitter receptor by interacting with a small hydrophobic pocket on the receptor protein, rather than with membrane lipids or intracellular proteins.

ACETYLCHOLINE RECEPTORS: NICOTINIC DEVELOPMENT

56.1 CONTROL OF m1 MUSCARINIC RECEPTOR GENE EXPRESSION. S. Pepeini, M.C. Snel, C.A. Harrington and N.J. Buckley. Wellcome Laboratory for Molecular Pharmacology, University College London, London WC1E 6BT, U.K.

G-protein coupled receptors are encoded by one of the most diverse gene families in the nervous system. As a step towards understanding the factors controlling GPR expression, we have determined the cDNA sequences of the muscarinic M1 and M4 promoters together with members of the muscarinic receptor gene family. RT-PCR and in vitro hybridisation reveal two waves of expression of the m1 gene in the embryonic rat brain. Around E12, there is a transient and widespread wave of m1 gene expression over wide areas of neocortical, the developing forebrain, mesencephalon and myelencephalon - only the forebrain expresses m1 receptors in adulthood. This transient expression is followed by a restriction of expression to those areas which express the gene in adulthood. Transcripts detectable in cortex (E16) and hippocampus (E18) co-occur with expression in the striatum which is not detected until E18: at this stage m1 is expressed over differentialed striatal cells but not over ventricular or intermediate zone cells. Receptor gene expression appears to precede cholinergic innervation. Thus it appears that adult patterns of expression are achieved by consecutive waves of activation and repression. In order to identify regulatory elements directing these patterns of expression we have isolated a genomic clone from a pBluescript II plasmid library and mapped the upstream regions of the m1 gene. Transfection of this cloned clone into a human neuroblastoma cells NB-OK-1 - a cell line that expresses an endogenous m1 receptor - followed by RT-PCR analysis using species specific primers demonstrated transcription of the rat m1 gene in transfected NB-OK-1 cells. 5RACE and SLIC procedures were used to gain more 5'UTR information. Hybridisation probes derived from RACE/SLIC sequences were then used to map the non-coding exons of the m1 gene and to characterise its promoter.


β3 is a member of the neuronal nAChR gene family. Its expression has been analyzed in the chick nervous system by Northern blot, in situ hybridization and immunohistochemistry during development. In contrast to other subunits such as α2 or β2 that are widely distributed, β3 expression is very restricted in the CNS. The β3 mRNA and protein have only been detected in the ganglion and amacrine cells of the retina.

To identify DNA sequences responsible for the spatiotemporal specificity of its expression, 3 kb of the 5' flanking region of the β3 gene were cloned and assayed for promoter activity by fusion to a reporter gene (CAT or lacZ) in transient transfections of primary cultures of neural cells (Matter-Sadzinski et al., 1992, EMBO J. 11, 4529-38). A 150bp fragment located just upstream from the transcription initiation site displays specific promoter activity and drives reporter gene expression in retinal ganglion cells. This suggests that the main regulatory elements are contained in this short region. Sequence analysis reveals the presence of classical promoter elements such as TATA and CAAT boxes, as well as several putative transcription factor binding sites. Disruption of one of them, an E-box (binding site for BHLH proteins), or its displacement relative to the CAAT box lead to a tenfold reduction in promoter activity, suggesting the involvement of a BHLH factor in the control of β3 transcription.

56.3 PHARMACOLOGICAL ANALYSIS OF α-BUNGAROTOXIN BINDING SITES IN THE DEVELOPING AND ADULT RAT SOMATOTAXIC CORTEX AND HIPPOCAMPUS. R.S. Branda*, D. Aceto and P.M. Leslie, Department of Pharmacology, University of California, Irvine, CA 92717.

Past studies of the developing rat brain have demonstrated a unique transient expression of [125I]-bungarotoxin (α-BTX) binding sites in the sensory regions of the cortex during developmental stages. In the present study we have also seen a transient expression of α-BTX binding sites in all developing regions of the brain. In order to determine whether this transient expression is associated with changes in the binding properties of the site with age, we have used quantitative autoradiography to examine changes in binding in specific brain regions. Our autoradiograms were analyzed by computer-assisted densitometry; quantitative results were obtained from the mean square deviation of the specific binding. Saturation studies revealed saturable [125I]-α-BTX binding sites in sensory cortex (SS1) and hippocampus of rat aged embryonic day 19, postnatal day 7 and adult. Animals were sacrificed and their brains removed, frozen in isopentane and cryostat cut out into 10-μm-thick sections. Sections were incubated with [125I]-α-BTX in the presence and absence of competing ligands, washed, dried and subjected to autoradiography. The resulting autoradiograms were analyzed by computer-aided densitometry to quantify relative binding density. In summary, no changes were observed in any brain region at any age. However, in both regions the following order of affinity was observed: α-BTX > <α-Cho > β-Cho > nicotine > cyanide. At E19 and P7, competition curves for agonist inhibition of [125I]-α-BTX binding displayed Hill slopes of 1±2, whereas slopes of <1 were observed in the adult. In contrast, antagonist binding curves exhibited Hill slopes of <1 at all ages. These data suggest that the appearance of positive cooperativity of agonist binding in the adult. This change in the binding profile of agonists may be associated with the downregulation of [125I]-α-BTX binding sites in the adult.

Supported by PHS grant 5R01NS20109.
466.1

Studies suggest that nicotine induces the release of dopamine (DA) and acetylcholine (ACh) through interactions at α3β2 and α4β2 nicotinic receptor subtypes, respectively. The chronic administration of nicotine increases nicotine-mediated [3H]DA release, but decreases nicotine-mediated [3H]ACh release from brain slices, suggesting that chronic nicotine modulates the effects of these receptors. To test this hypothesis, CRNAs for α3 and β2 or α4 and β2 subunits were co-injected into Xenopus oocytes and nicotine-activated currents were measured before and after 24 hrs incubation with nicotine using the 2-electrode voltage-clamp technique. Currents in oocytes expressing α3β2 receptors were not affected significantly by 24 hr incubation with nicotine; currents increased 6.8-fold, 5.9-fold and 5.2-fold following incubation with 0, 20 and 100 nM nicotine, respectively, due to increased receptor expression. In contrast, α4β2-expressing oocytes had a reduced response to nicotine; current in control oocytes increased 5.8-fold, whereas current in oocytes incubated with 20 nM nicotine increased 2.2-fold and with 100 nM incubation, no increase was noted. This reduced response to nicotine reversed after 90 min incubation in medium lacking nicotine indicating α4β2 receptors were expressed, but desensitized. Results suggest that the differential effect of chronic nicotine on the release of DA and ACh may be due, at least in part, to differences in the desensitization of the two receptor subtypes that mediate the release of these neurotransmitters. (Supported by grants #0411 from the STRC, Inc. and #AA09212 from the NIH.)

466.2
NICOTINE-INDUCED DESENSITIZATION OF RAT NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT COMBINATIONS EXPRESSED IN XENOPUS ЛаЕВИС ООКЙТЕС. C.R.T. Vilañ, J.A. Lusà, M.G. McNarine, and E.L. Ochoe, Section of Molecular and Cellular Biology, and Department of Pediatrics, University of California at Davis, Davis, CA 95616.

Chronic administration of nicotine up-regulates rat neuronal nicotinic acetylcholine receptors (nAChRs). A key hypothesis that explains up-regulation assumes that nicotine induces desensitization of receptor function. This is correlated to behaviorally expressed tolerance to the drug. The present experiments were conducted to obtain information on the nicotine-induced desensitization of neuronal nAChR function, a less understood phenomenon, as compared to that of the muscle and Torpedo receptors. X. laevis oocytes were injected with nRNA encoding receptor subunits α2, β2, or α2β2 in pairwise combination with the β2 subunit. The responses to various concentrations of ACh or nicotine were analyzed by the two-electrode voltage clamp technique. α2β2 is the predominant form in the rat brain that also undergoes up-regulation (Plomp, et al., 1992). We found that nicotine was more potent than ACh (EC50: 0.3 μM vs. 6 μM) in the α2β2 combination and we observed a depression of the maximum attained response at concentrations higher than 20 μM nicotine, a clear indication of nicotine-induced desensitization. Inactivation of the response (calculated as a single exponential decay) was significantly faster for [ACh] than for α2β2. We constructed UV curves at different concentrations of nicotine and the results suggest that blockade is not the mode of receptor inactivation at high nicotine concentration. By patch clamp analysis, α2β2 has been shown to have three conductance states (Chameau, et al., 1992). Since one or more of these states could differentially contribute to the observed desensitization, experiments are in progress to analyze desensitization kinetics at the single channel level. Taken altogether, these results suggest that the α2β2 combination is desensitized by nicotine. This research was supported by NIH grant NS32294 and in part by funds provided by the Cigarette and Tobacco Surplus Fund of the State of California through the Tobacco-Related Disease Research Program of the University of California, Grant Number 07-0098.

466.3

Chronic nicotine exposure in tobacco smokers or experimental animals is known to cause an increase in brain nicotine binding sites and to cause accumulation of chronically desensitized receptors. Acetylcholine receptors of the same (α4)β2)3 subunit composition as the predominant subtype of brain nicotinic receptor with high affinity for nicotine have been expressed in a permanently transfected fibroblast cell line. Chronic exposure of these cells to nicotine, other agonists, or a channel blocker, is shown to result in an increase in receptor amount, indicating that nicotine induced upregulation reflects properties of the α4β2 receptor protein which can be expressed in transfected fibroblasts, rather than an adaptive response unique to the neurons in which these pathways are normally expressed. The nicotine concentration-dependence, time course, and extent were similar to those reported for ligands in brain, suggesting that this intrinsic property could account for the upregulation observed in brain. The mechanism of nicotine-induced upregulation is shown to not require ion flow through the receptor and to involve a decreased rate of receptor turnover.

466.4

The α5 subunit of the chick neuronal nicotinic acetylcholine receptor (α5nAChR) family is present in many cholinergic neurons (Bullivant, Berg and their colleagues). Emerging evidence indicates that the α5 subunit can participate in functional nicotinic channels by coassembly with other α and β subunits in heterologous expression systems (see Abstract by Rambuyt-Lewis et al., 1993). In view of this data we have examined the role of α5 subunits in native neuronal nAChRs.

The properties of αCh-gated macroscopic and single channel currents were assayed in embryonic chick sympathetic neurons treated with control or α5 antisense oligonucleotides. Two different antisense oligonucleotides against different regions of the α5 subunit are used and produced identical results: First, α5 deletion alters the dose response curves to ACh, its cytotoxicity and nicotine with changes in the apparent affinity for agonist. Thus α5-minus neurons have a higher affinity receptor (EC50s=150 vs. EC50s=450 μM) composed with α5 minus neurons. These results are consistent with previous findings that inclusion of α5 subunits in heterologously expressed α5β2 nAChRs decreases agonist affinity. In addition, the deletion of the α5 subunits changes the profile of α5β2 channel subtypes. Two of the three classes of channel expressed in control neurons are deleted and new channels of 14pS and 6pS are expressed by sympathetic neurons. These results indicate that the α5 subunit participates in native sympathetic neuronal nAChRs altering both agonist affinity and conductance. (Supported by NHLBI8071 to LR)
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MUTATIONAL ANALYSIS OF NOVEL RESIDUES IDENTIFIED WITHIN THE BINDING SITE OF d-TUBOCURARINE OF TORPEDO ACETYLCHOLINE RECEPTOR: Y. Xia, D.C. Chiaia and J.B. Cohen
Departement of Molecular Biology, University of California, San Diego, CA 92037.

The competitive antagonist d-tubocurarine (dTC) binds to the two agonist binding sites of the nicotinic acetylcholine receptor (nAChR) from Torpedo electric organ and mouse skeletal muscle with significantly different affinities.

The high-affinity site is saturated at the -α and α-ε subunit interfaces, respectively. Our photo labeling studies using [3H]dTC identified previously two non-α residues: Y235 and F237. Here we report the identification of another high-affinity (H-α) binding site (H-αTC; Y111 and Y117). The tyrosine in the α-subunit of muscle nAChR corresponding to Torpedo Y117 has been shown to be important for the selective binding of dimethyl-TC (S. Sine, PNAS 90: 9346-9400, 1993). In the aligned Torpedo nAChR sequence, Tyr117 is conserved, whereas the equivalent residue Y111 in the ε subunit is R113. This difference may also contribute to the difference in dTC binding affinities between the two sites. To examine the role of these two residues in dTC binding, we made Y111R and R113Y mutant receptors. When expressed in Xenopus oocytes, both mutant receptors showed similar densities of [3H]-bungarotoxin (oBgt) binding and amplitudes of ACh-induced currents as wild type receptors. Competition studies of dTC against the initial rate of [3H]-oBgt binding to both whole oocytes and oocyte membrane reveals that Y111R mutation causes 3-fold decrease of dTC affinity for the high-affinity binding site, while the R113Y mutation increases dTC affinity for the low-affinity binding site 2.3-fold. Functional consequences of these mutants in terms of changes of agonist/antagonist sensitivities will be assessed by using the two-electrode voltage-clamp technique.


Cells of the SH-SY5Y human neuroblastoma express two types of nAChR, nicotinic α-bungarotoxin (Bgt) binding sites (nBgt) and ganglia-type α6β4γ2δ nAChR products of at least five human nAChR subunit genes (α3, α5, α7, β2 and β4). Here we show that transfection of SH-SY5Y cells with rat α7 constructs dramatically increases [3H]-labeled Bgt (1-Bgt) binding in SH-SY5Y cells without affecting ganglia-type α6β4γ2δ nAChR function. Cytosporin sensitivity of this effect, which is observed only in α7 binding to control SH-SY5Y cells, suggests that transgenic rat α7 subunits may assemble in a cytoplasm-dependent manner as homologomers, whereas assembly of native α7-containing nAChR α7 does not have such a dependency. Preliminary studies indicate that transfection of SH-SY5Y cells with antisense α7 constructs produces a dramatic loss in I-Bgt binding, again without effects on ganglia-type α6β4γ2δ nAChR function, suggesting that human α7 subunits contribute to native nAChR but not ganglia-type α6β4γ2δ nAChR in SH-SY5Y cells. Other studies demonstrate that adult muscle nAChR epsilon ternary expression in BC-1-1 cells rescues nAChR from loss of function and I-Bgt binding when cells are subjected to drug treatments that mimic the motor neuron innervation-induced loss of fetal nAChR gamma subunit expression as seen in developing muscle in vivo.


Changes in cyclic AMP (cAMP) levels in differentiating muscle cells influence the expression of muscle nicotinic acetylcholine receptors (nAChRs). We assessed effects of 8-(4-Chlorophenyl)-cAMP (CPT-cAMP), a cyclic AMP analogue, on nAChR expression in BC3H-1 mouse muscle cells. CPT-cAMP treatment of cells maintained in a myoblast-like state (where cells are grown in medium containing 10% horse and 5% fetal calf sera) inhibited the usual slow, time-dependent accumulation of nAChR. Conversely, for "differentiation" of cells where a typical 2-fold increase in nAChR expression is rapidly induced by at least 2 days of growth in 1% fetal calf serum-supplemented medium, CPT-cAMP exposure produced an additional up-to-2 fold augmentation in nAChR expression. These divergent effects of CPT-cAMP treatment are reflected in nAChR function, numbers of nicotinic radioligand binding sites on the cell surface or in total particulate fractions, and levels of nAChR subunit mRNA. These findings suggest that effects of activation of cAMP-dependent signaling in muscle cells in vivo might also vary as a function of cell differentiation state, acting to inhibit nAChR expression in myoblasts but to enhance the induction of nAChR expression after myotube formation.

646.9 SUBUNIT COMPOSITION DISTINGUISHES MULTIPLE CLASSES OF ACETYLCHOLINE RECEPTORS IN THE SAME NEURONS. W.G. Conroy*, and D.B. Berg. Dept. of Biology, UC San Diego, La Jolla, CA 92039.

The role of subunit composition in regulating neuronal acetylcholine receptors (AChRs) is only beginning to be elucidated. Chick ciliary ganglion neurons express five known neuronal nAChR genes. Three of these products (α3, β4, and σ5) are present in synaptic-type receptors that bind mA35 (mA35-AChrs). A fourth (α7) is present in a second class of receptors that bind α7-bungarotoxin (α7-Bgt-AChrs). Immunoprecipitations with subunit-specific mAbs now show that the fifth gene product (β2) is present in about 28% of the mA35-AChrs which correspond to α7-Bgt-AChrs. Immunoblot analysis confirms that the β2 gene product is associated with α3, β4, and σ5, but not with mA35. The increased presence of β2 subunits in mA35-AChrs containing β2 subunits. Using a sensitive solid phase assay we report a new population of putative receptors that bind both mA35 and mA7. These mA7 receptors are developmentally regulated, and are also found in dorsal root ganglia but not in brain or retina. Analysis with subunit-specific mAbs provides no evidence for receptors containing any other subunit. AChR gene products known to be present in ciliary ganglia. The muscle AChR of mammalian skeletal muscle binds both mA35 and mA7, and RNA expression studies indicate a small amount of α7 transcript in the ganglia. Immunoblot analysis, however, fails to detect α7 protein in the receptors. The results raise the possibility that mA7 receptors are composed of novel gene products yet to be described. (NS14201, NS25916, & TRDRP)

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Subunit functions and interactions with other cholinergic receptors are poorly understood in the case of neuronal nicotinic acetylcholine receptors (AChRs). To address these issues, we have expressed three AChR genes transfected into HEK-293 cells. These subunits make up the major class of synaptic-type AChRs that binds mACh 35 on chick ciliary ganglion neurons. Immunoblots of extracts from transfected cells confirm the expression of gene products that co-migrate with the subunits from native AChRs. Sucrose gradient analyses of extracts demonstrate that cells transfected with a3/b4/a5 (but not a3/b4) contain larger amounts of assembled components that bind mACh 35, immunoprecipitate with anti-a3/b4 mAbs, and sediment in the 100S class. Furthermore, the similar amounts of such components in the three kinds of transfections, a5/b4 cells express by far the most mACh 35 binding sites on the cell surface. Surprisingly, transfections with a5 alone produce large amounts of surface sites for mACh 35 (a3 and a4 alone do not), but immunoprecipitations indicate that the a5 species cannot alone account for the large number of sites on a5/b4 cells. The results identify a new candidate neuronal AChR and suggest that a5 subunit may play a key role in regulating receptors to the surface. (NS12601, NS52516, AHA93-72)

DIFFERENTIAL EFFECTS ON CALCIUM LEVELS IN NEURONS CAUSED BY NICOTINIC AND MUSCARINIC RECEPTORS. M.M. Rathouz*, S. Vlavaraphavan, and D.K. Berg. Dept. Biol., UC San Diego; La Jolla, CA 92093.

Intracellular calcium levels ([Ca]i) in neurons can be elevated by either nicotinic or muscarinic receptors. Chick ciliary ganglion neurons have both. The nicotinic receptors increase [Ca]i via their calcium permeability and activation of voltage-dependent calcium channels. We report here that a3/b4-type muscarinic receptors on the neurons have different effects on [Ca]i.

Activation of the muscarinic receptors stimulates phospholipase C and increases IP3 levels. As the IP3 turns over, it is blocked by DAMP as expected for M3 receptors. Fluorimetric measurements in fura-2-loaded neurons indicate that the increase in the receptor increases [Ca]i in an oscillatory manner that is blocked by DAMP, depends on extracellular calcium, but remains after emptying caffeine-sensitive calcium stores. These findings indicate that M3 receptors can oscillate through the opening of voltage-dependent calcium channels.

ACh at 50 pM activates both the M3 and nicotinic receptors. Additional experiments indicate that the M3 receptors induce calcium oscillations in a manner that can be blocked by a selective blocker of M3 receptors acting through IP3 to produce the oscillations.

The distinctive changes in [Ca]i produced by the two receptors may allow a single neurotransmitter to modulate multiple calcium-dependent events independently in a neuron. (NS12601, NS25916, & TRDRP)

ALTERATIONS IN CYCLOSPORIN-A SENSITIVITY OF a7 HOMOMERIC NICOTINIC RECEPTOR EXPRESSION BY MUTATIONS WITHIN TRANSMEMBRANE DOMAINS. Santosh A. Helekar*, Hong Dong and Jim Patrick. Dev. of Neuroscience, Baylor College of Medicine, Houston, TX.

Synthesis of oligomeric integral membrane receptors such as the ligand-gated ion channels may depend on the activity of folding enzymes and molecular chaperones. We showed previously that blockade of a folding enzyme, the prolyl isomerase cyclophilin (Cyp), with cyclosporin A (Csa) caused a dose-dependent reduction in the function of functional nAChR receptors in Xenopus oocytes. We now report a second effect of Csa on the small number of residual receptors that are assembled in the presence of this compound. We observed that, in addition to the apparent reduction in receptor expression caused by this increase in Ca2+ entry, run down induced by Csa alone was doubled, as was the wild type. However, in contrast to the wild type receptor, Csa produced a significant enhancement of the level of expression of functional nAChR receptors. These data indicate that Csa on a7 nAChR nicotinic receptors, namely on receptor expression and on receptor run down are distinct and unrelated. The mechanisms underlying the run down effect of Csa may be different from the Csa-induced enhancement of receptor expression as it can be reversed by the substitution of a glycine residue for a histidine on the transmembrane domain I. Supported by grants from NIH (JP) and a MDA fellowship (SAH)

POSTTRANSCRIPTIONAL REGULATION OF NEURAL AC CHOLINE RECEPTOR GENES DESTINED FOR THE CELL SURFACE. R. Rothbaur, S. Romano, and B. Berg. Dept. of Biology, UC San Diego, La Jolla, CA 92093.

Mechanisms controlling receptor accumulation on neurons are largely unknown. We have found that the acetylcholine receptor (AChR) a4 and b2 genes, permit an examination of this process. We find that forskolin in 24 hours stimulates a face increase in cell surface AChR and a 3-fold increase in the abundant intracellular receptors. These increases are partially blocked by H89 and mimicked by b-hromo- cAMP, implicating PKA activation. Our results show that the face increase and part of the surface increase depend on protein synthesis and correlate with increases in receptor transcripts. When protein synthesis is blocked, however, the increase doubles selectively the number of surface AChRs, suggesting that a CAMP-dependent process either helps target receptors to the cell surface or stabilizes them there. Okadaic acid, which inhibits protein phosphatases 1 and 2A, selectively blocks the large forskolin effect on surface receptors but not by effect on intracellular receptors. Okadaic acid does not decrease total protein synthesis or AChR transcript levels in forskolin-treated cells, but does decrease the levels of AChR proteins, possibly by enhancing turn over. The finding that forskolin and okadaic acid have opposite but specific effects on the accumulation of surface AChRs suggests that multiple phosphorylation sites on the AChR are involved in receptor assembly, transport, and stability. (NS12601, NS25916, & TRDRP)


Fifteen nicotinic ACh receptor (AChR) genes have been identified; a subset of these (a2-8, a2-4) are expressed in the nervous system and are therefore considered neuronal genes. Using RNasea protection assays we demonstrate that embryonic chick skeletal muscle expresses both a7-type AChR genes (a4, a5, a7, and b4) in developmentally regulated patterns. Of the four, a7 mRNA is relatively abundant and reaches a maximum level in embryonic day 8 (E8). Like a1, a7 mRNA levels increase over development, peaking at E8-E11, and fall dramatically at E17. Immunoepitope studies confirm the presence of a7 transcripts in myotubes both in vivo and in culture. Immunoprecipitations and immunoblot analysis using subunit-specific monoclonal antibodies reveal that this muscle; the developmental pattern of a7 protein expression parallels that of a7 mRNA. Sucreose gradients demonstrate that the a7 proteins present in a 10S species, a size expected for an AChR. The a7-containing component binds a-hungarotoxin but does not contain a3 subunits, indicating that the two a-type genes product segments separately during assembly. The finding of a putative neuronal AChR in embryonic muscle indicates that the tissue-specific division of AChR gene expression is less categorical than previously recognized and raises the possibility that neuronal AChRs participate in muscle development. (NS12601 and NS25916)


We have expressed a variety of mammalian nicotinic receptors in Xenopus oocytes. The a7, a4/b2, combination gave a current of 300nA ± 122nA (n = 7) in response to 10μM acetylcholine (ACh), with a t50 of desensitization of 51.4 ± 3.0ssecs. In the presence of 5HT there was a dose dependent decrease in Ic50 (5HT) of the receptors in 3.2ssecs. We now report a second effect of 5HT on the small number of residual receptors that are assembled in the presence of this compound. We observed that, in addition to the apparent reduction in receptor expression caused by this increase in Ca2+ entry, run down induced by 5HT alone was doubled, as was the wild type. However, in contrast to the wild type receptor, 5HT produced a significant enhancement of the level of expression of functional nAChR receptors. These data indicate that 5HT on a7 nAChR nicotinic receptors, namely on receptor expression and on receptor run down are distinct and unrelated. The mechanisms underlying the run down effect of 5HT may be different from the 5HT-induced enhancement of receptor expression as it can be reversed by the substitution of a glycine residue for a histidine on the transmembrane domain I.
Molecular regulation of human $\alpha_7$ nAChR gene expression. L.M. Montezinos, M. Cogswell-Smith, and T. Giudisco. Neuroscience Research, 49(3), 2003. Previous reports of $\alpha_7$ nicotinic acetylcholine receptor (nAChR) gene expression have suggested augmented expression of the gene regulatory mechanisms with differences between transcriptional and steady state levels of the RNA reported during chicken development. We report here the regulation of $\alpha_7$ in the human neuroblastoma cell line, IMR32, in response to NGF and PMA, agents which are known to alter the expression of various genes. Three transcripts of 6.0, 3.5, and 2.4 kb were observed for $\alpha_7$. An approximately 2-fold increase in the steady state levels of RNA of all transcripts was detected following 24 hr treatment with either NGF or PMA. To determine the kinetics of $\alpha_7$ gene induction, IMR32 cells were treated with NGF for 24 hr and 24 hr RNA was isolated and steady state levels were determined. An increase in steady state levels of RNA can result from increased stability, translation, or both. In order to detect alterations in transcriptional activity, cells were treated with NGF or PMA for 24 hr and the nuclei were isolated for RNA runoff experiments. The stability of the $\alpha_7$ RNA is decreased in a fashion similar to that observed in a companion Drosophila RNA report. The 3UTR region of mRNA sequences rich in A and U nucleotides, which seem to function in stability, translation, and localization of the RNA. Using RNA footprints to analyze the 3UTR of the human $\alpha_7$ cDNA no AUUUA sequences, a motif previously shown to regulate RNA stability, were found, however a large stem loop structure was predicted. In a 9-ribop, ATATCTCA, +2-7 are identical in rat, suggesting this may be a motif for RNA binding proteins (RNP's). Studies are underway to determine whether RNP's are present which bind to the human $\alpha_7$ 3 UTR and whether the 3' UTR from rat or other human subunits can compete for the proposed binding protein.

Neuronal nicotinic acetylcholine receptor $\alpha_4$ mRNA consists of three different transcripts in rat brain: Northern determination. J. D. Y. D. Morgan, D. M. Copeland, Pharmacol. and Therap., USF College of Medicine, Tampa, FL 33612. The initial report describing the neuronal nicotinic receptor $\alpha_4$ RNA identified 3 different messengers believed to represent alternative splicing of a single transcript (Cell 48:965, 1987). Although $\alpha_4$ is the most widely expressed subunit mRNA in rat brain, the 3 species of $\alpha_4$ transcripts have not been well described with respect to their abundance ratios in different brain regions. In this study, the sizes of $\alpha_4$ mRNA were determined by Northern hybridization with radiolabeled plasmid DNA for $\alpha_4$ -1 or $\alpha_4$- II fragment of $\alpha_4$ -1. Quantification was conducted by densitometry of X-ray film. Three transcripts homologous to $\alpha_4$ -1 cDNA were found in all brain regions tested. The sizes of the $\alpha_4$ gene transcripts were approximately 2.6, 4.6, and 6.0 kb. The ratios of the 2.6:4.6:6.0 kb transcripts were 1:0.6:0.11 in basal forebrain; 1:0.1:0.28 in striatum; 1:0.04:1.0 in corpus callosum; 1:0.1:0.04 in cerebellum; 1:0.1:0.04 in midbrain cortex; 1:0.2:0.10 in midbrain; 1:0.7:0.13 in hippocampus; and 1:0.0:0.34 in thalamus and hypothalamus. Further, all 3 bands diminished in parallel with increased hybridization stringency, indicating a similar degree of homology to the $\alpha_4$ -1 probe. This study demonstrates that there are 3 alternatively processed transcripts of the neuronal nicotinic acetylcholine receptor $\alpha_4$ gene, and the relative abundance of these transcripts differs in different brain regions. (DGM is an Established Investigator of the American Heart Association. Supported by grant #0411 from the SRC, Inc.)

A novel in situ double-labeling procedure to study the expression of proteins and messages. K.P. Chia, S.A. Berman, T. Sullivan and S. Burzynski. Labs. of Molecular Neurosci., Howard Hughes Med. Inst., Johns Hopkins Univ. School of Med., Baltimore, MD 21205. Nicotinic acetylcholine receptors (AChRs), the hallmark of neuronal-muscular junctions, express multiple receptor subunits which can be regulated by many signals. In our investigation of these phenomena, using cultured muscle cells and neurons isolated from developing embryos, it is frequently necessary to image more than one molecular response simultaneously. We devised a novel double-labeling procedure to detect the expression of two different signals, e.g. 2 different mRNA species or a protein together with its corresponding mRNA, in the same cell. This method employs a separate emulsion-coated slide to detect the radioactive signal, while the other signal is detected directly on the same slide. We used this method using the combination of 3'-labeled b-horagonin toxin to detect digoxigenin-labeled probe, or the combination of 5'-labeled cDNA probe together with digoxigenin-labeled probe. After exposure, the emulsion-coated slide was separated from the coverslip containing the cell sample and then developed. We used the coverslips coated with silver to detect the digoxigenin-labeled probe. After color development, the coverslip carrying radioactive signal was recombined with the coverslip carrying the colored signal and the coverslip was allowed to dry at room temperature. This procedure allows for the detection of distribution of AChR protein as observed by silver grains and AChR subunit message as detected colorimetrically on the same cell. Similarly, our procedure allows for the detection of two different messages in the same cell.
FREQUENT, LONG-LIVED SPONTANEOUS AND MONO LIGANDED OPENING OF ACETYLCHOLINE RECEPTOR WITH A L521C INTERFACE

An important functional property of muscle-cell acetylcholine receptors (AChR) is that they should remain closed in the absence of transmitter. It is well known that AChRs are not spontaneously active, but such events occur at a very low frequency and have extremely brief lifetimes. When expressed in HER293 cells and exposed to 100 nM ACh, the receptor displayed a high rate of spontaneous opening, which did not persist when [3H]Acetylcholine (ACh) was added. An important characteristic of this high rate of spontaneous activity is its susceptibility to cholinergic interventions. The use of nicotinic cholinergic agonists to activate the receptor at a high rate of spontaneous activity is consistent with the apparent low levels of neurotransmitter released at the neuromuscular junction. The results suggest that unliganded muscarinic receptors the energy barrier to gating is lowered, i.e., there is a destabilization of the gate.

RUDELS B. A., et al., J. Biol. Chem. 262, 4978-4986, 1987. In the presence of mAChR, we observed a 0.9-fold decrease in nAChR single-channel amplitude at -100 mV, whereas Fab 387 completely inhibits channel conductance. The labeling studies also suggest that the mAChR and Fab 387-nAChR complex is not a resting state, hence it is not clear if the result is due to a change in the functional state of the receptor. The mAChR has been previously shown to decrease α-BTX and ACB binding to NACHRs (Mihovilovic and Richman, J. Biol. Chem. 259: 15011-15015, 1984). Our patch clamp studies demonstrate that both mAChR and its respective Fab fragment completely inhibit channel opening. The labeling data obtained with the mAChR 247-nACHR complex suggests that mAChR can transition from a resting state to a desensitized state without entering the active state.

MUTATIONS AT THE LIPID-PROTEIN INTERFACE OF THE ACETYLCHOLINE RECEPTOR AFFECT CHANNEL GATING

L. J. Rudeles, D. H. Bueler, and M. G. McIntosh* Section of Molecular and Cellular Biology, University of CA, Davis, CA 95616.

We have investigated the interactions of monoclonal antibodies (mAb) with the Torpedo californica acetylcholine receptor (nAChR). Using patch clamp analysis, anti-Torpedo monoclonal antibodies were tested to determine their effect on nAChR function. In addition, 125I-labeled nAChR were incubated with 3-[3H]Acetylcholine (3-[3H]ACh) to determine the absence and presence of agonists. Agenist-induced desensitization of the nAChR reduces T-cell labeling and serving as a probe of the receptor states. The mAChR 247 has been shown to selectively block one of the α-6bontasteggin (α-BTX) sites at the nAChR for acetylcholine (ACh) (Mihovilovic and Richman, J. Biol. Chem. 259: 15011-15015, 1984). Our patch clamp studies demonstrate that both mAChR and its respective Fab fragment completely inhibit channel opening. The labeling data obtained with the mAChR 247-nACHR complex suggests that mAChR can transition from a resting state to a desensitized state without entering the active state.

Identification of Drosophila loci whose products show structural similarity to the vertebrate nicotinic acetylcholine receptor. B.A. Chase*, Dept. of Biology, Univ. of Nebraska-Omaha, 600 & Dodge Sts., Omaha, NE 68182.

The identification of multiple α- and β-like invertebrate nAChR subunits has suggested that there may be considerable heterogeneity in neuronal nAChRs. To identify potential Drosophila nAChRs or other molecules sharing structural similarity to the vertebrate nAChR, mAb probes to the vertebrate nAChR were previously used to identify putative orthologs in Drosophila (Chase et al., 1987). However, the mAb specifically cross-reacted with neuronal cholinergic function. To isolate the genes that encode the antigen(s) recognized by this mAb (27.14.16.42), a lambda-ZAP expression vector library has been screened and three cDNAs isolated. The cDNAs have little homology to known Drosophila nAChR genes. Chromosomal hybridization has also indicated that these cDNAs do not derive from loci encoding known Drosophila nAChRs. Thus, the cDNAs identified by mAb 27.14.16.42 derive from loci that appear to be expressed in a pattern consistent with this mAb's cross-reactivity, but do not encode known nAChR subunits. As this mAb recognizes structural features of the β-subunit of the vertebrate nAChR that are presumably shared by the cross-reacting Drosophila antigens, the loci identified here may encode novel β-like subunits of a nAChR, or other proteins, perhaps members of the superfamily of structurally related neurotransmitter receptors including nACH, GABA-A, and glycine receptors. The cDNAs encode three proteins of 38, 42, and 46 kilodaltons, respectively. Two of the three cDNAs have structural similarity to the vertebrate nAChR. The third cDNA has unique features that may indicate a novel nAChR subunit.
466.29
Transcription of neuronal nicotinic acetylcholine receptor subunit genes in thymic epithelial and thymic lymphoid cells.

466.30
UPSTREAM SEQUENCES REQUIRED FOR CONTROL OF αR EXPRESSION IN DROSOPHILA. L. C. Yang, Y. He, and T. Schmidt-Glenewinkel. Department of Biological Sciences, Hunter College of CUNY, New York, NY 10021.

The αR genes encode a non-α-like subunit of one of the neuronal nicotinic acetylcholine receptors in Drosophila. We are interested in studying the cis-acting elements and trans-acting factors required for temporal and spatial expression of the genes encoding the acetylcholine receptor subunits. 5'-flanking sequences and intron sequences were cloned into the P-element containing vectors HZ50PL and CZ20XN generating either enhancer fusion or transcriptional fusion lacZ genes respectively. P-element-mediated germ-line transformation and establishment of balanced transformed stock was carried out by standard procedure. Frozen sections of whole flies or of heads were briefly fixed and examined for expression of lacZ fusion genes using either X-gal or an antibody against β-galactosidase. "Enhancer fusion" using about 4kb of 5'-upstream sequence show spatially correct expression in Drosophila heads while addition of 3kb of upstream sequence showed no expression. Spatially correct expression of "transcriptional fusion" constructs required additional downstream sequences. Constructs using a series of 5' and 3' deletions as well as internal fragments allowed us to define several upstream elements required for expression in Drosophila.

466.31
CHARACTERIZATION OF TWO TIGHTLY LINKED DIVERGENT PROMOTERS AND A SLIGHTER ELEMENT LOCATED BETWEEN THE RAT j4 and α3 NICOTINIC ACETYLCHOLINE RECEPTOR GENES. B. T. Boyd. Department of Pharmacology and The Neuroscience Program, The Ohio State University College of Medicine, Columbus, Ohio.

In order to understand the transcriptional regulation of neuronal nicotinic acetylcholine receptors by cell contact and electrical activity, it will be first necessary to identify DNA elements that control the expression of neurons of this family and to identify factors required for the expression of these genes. Toward this goal we have identified two promoter regions in the j4-α3 intergenic region. One region is close to the j4 gene downstream of exon 6 and has strong promoter activity in both orientations; the other is close to the α3 gene, in the j4 gene coding region. The basis of the sense and antisense promoter constructs indicated the bidirectional promoter activity demonstrated within the j4 gene is due to two very closely linked promoters that may share common elements. The functional assay for the antisense promoter is not clear. The regulated production of antisense RNA could be involved in the regulation of the j4 gene at the transcriptional or translational levels. Opposite strand RNAs could also affect the expression of the α3 gene from the upstream promoter. A region with putative silencer activity was also found near the upstream promoters. DNA-binding protein interactions within this element have been studied. The level of a protein in PC12 cells that binds to an A+T rich region in the silencer was shown to be regulated by NGF. This work supported by AHA (Ohio Affiliate), Bremner Foundation (Columbus, Ohio), and NIH Grant NS29746.

466.32
CLONING AND CHARACTERIZATION OF THE HIPPOCAMPAL α7 NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR FROM HUMAN POSTMORTEM BRAIN. J. Loge, C. Debing, C. Antle, C. Breese, M. Hall, C. Adams, B. Friedman and S. Leonard, VA Medical Center and University of Colorado Health Sciences Center, Denver, CO 80262.

The full length sequence of the human α7 neuronal nicotinic receptor subunit was obtained from human hippocampus. A partial clone was obtained by screening a human hippocampal library. The amino terminus region of the cDNA was obtained with a 5' RACE clone generated from hippocampal RNA. Hippocampal α7 appears nearly identical to that cloned from the neuroblastoma cell line SH-SY5Y (X. Peng et al., 1994). Sequence obtained from PCR of cDNA suggests multiple exon boundaries. Northern analysis of human cingulate and hippocampal mRNA showed variation in size compared to message in the neuroblastoma cell line and rat brain. Chemical cross-linking of α7-bungarotoxin to membrane preparations of human and monkey hippocampus and whole synaptic brain revealed two principle protein species, one only of which was competed with nicotine and methyllycaconitine, in situ hybridization with a probe for the cytoplasmic loop of α7 showed labeling of a small population of cells in the dentate hilus, consistent with the localization of α7-bungarotoxin binding in control and schizophrenic brain tissue. Control and schizophrenic post-mortem brain was screened for polymorphisms by RFLP analysis and PCR.

466.33
EFFECT OF METHYLLYCACONITINE AND RELATED ALKALOIDS, IN VITRO AND IN VIVO, AT THE α-BUNGAROTOXIN BONDING SITE IN RODENT BRAIN. Y. D. Rollins1, M. Hall, K. L. Hemann, P. Dobelle, J. P. Walcotte2, J. M. Roper3, and S. Leonard4. 1Dept. of Pharmacology, UCHSC, and Medical Research, VAMC, Denver, Colorado 80262. 2Department of Anatomy & Neurobiology, Colorado State Univ., Fort Collins, CO.

Schizophrenia is partially characterized by an auditory gating deficit, an abnormal electrophysiological response to repeated auditory stimuli. It has recently been shown that gating deficit can be transiently reversed by nicotine. Additionally, a gating deficit can be produced in the use of a pharmacological manipulation which block a subset of the neuronal nicotinic receptors, the α-bungarotoxin binding receptor, α7. Both α-bungarotoxin and methyllycaconitine (MLA) halted intracranial electroencephalogram, induce a loss of gating of auditory evoked responses as measured by evoked potential recording in the unanesthetized animal with surface and depth (CA1) electrodes. We have compared the efficacy of related nicotinic agonists, with the amine MLA and MLA derivative containing the poisonous plant Research Lab, Logan, UT, to compete with α-bungarotoxin binding in rat brain membranes and find one of these compounds. 14-deacetylmatucaline (14-DN), has a higher affinity for the α7 receptor than does either α-bungarotoxin or MLA. In whole brain homogenates, 14-DN competes 112I-α-bungarotoxin with a Ki = 50 × 10^-12 M while MLA competes with a Ki of 6.6 × 10^-10 M. This affinity order 14-DN > MLA > daltexine found in brain membranes is similar to the potency order of these compounds in blocking transmission at the neuromuscular junction in an in vivo lard muscle preparation. These compounds will also be tested for their potency in blocking auditory gating.

466.34

At least five human mαCβR subunit genes (α7, α5, α3, β2 and β4) and two type s of αCβR are expressed by cells of the SH-SYSY human neuroblastoma. One class of mαCβR corresponds to nicotinic α-bungarotoxin (Bgt) binding sites (mBgt) which bind [125I]-labeled Bgt (1-Bgt) with high affinity and appear to contain human α7 subunits based on sense and antisense cDNA expression studies. SH-SYSY cells also express ganglion-type αCβR, which bind [3H]Acetylcholine with high affinity, mediate Bgt-insensitive but neurotoxin-sensitive cation and "Ca" influxes, and seem to contain human α3 and β4 subunits based on functional pharmacological profiles. To address questions remaining regarding the full subunit composition of ganglionic mBgt and αCβR, we have undertaken a series of affinity purification and immunochemical studies of the αCβR subtypes in SH-SYSY cells. Antibodies raised against unique peptide sequences of αCβR subunit cytoplasmic domains exhibit subunit specificity and interact with SH-SYSY cell membrane proteins on Western blots. Bgt-based affinity purification yields material that specifically binds 125I with high affinity and is recognized by anti-α7 peptide-specific antibodies, consistent with the presence of α7 subunits in mBgt.
ACETYLCHOLINE RECEPTORS: NICOTINIC MOLECULAR BIOLOGY

**466.35**

**MAPPING NEURONAL BUNGAROTOXIN SENSITIVITY ON NEURONAL NICOTINIC RECEPTOR ALPHABETA SUBUNITS**

C.W. Luebic*, F. Madix and S.C. Harvey. Molecular and Cellular Pharmacology, University of Miami, Miami, FL 33101.

Neuronal nicotinic acetylcholine receptors (nACHRs) can be expressed in Xenopus oocytes upon injection of cRNAs encoding various combinations of α and β subunits. The α3β2 subunit combination is sensitive to neuronal bungarotoxin (NBT; 100nM) blockage (98.0±1.9% block) while α2β2 is insensitive to NBTr. Previous work has shown that determinants of NBT sensitivity on α3 are localized to three distinct sequence segments (84-121, 121-151, 195-215), and that gln198 of α3 (pro in α2) plays a role in determining NBT sensitivity. We have made a series of mutations within these regions of α3, changing residues from what occurs in α3 to what occurs in α2. We assay the NBT sensitivity of mutant α3 subunits in combination with β2 upon expression in Xenopus oocytes. Changing thr143 of α3 to lys as in α2 (T143K) results in a loss of NBT sensitivity (7.7±4.6% block by 100nM NBT). Changing amino acid residues 159-166 of α3 as a group (VLIGSSMN) to what occurs in α2 (EQMTIVD) has a modest effect on NBT sensitivity (80% block by 100nM NBT). Amino acid changes in α3 that had no effect on NBT sensitivity include K87L, Q101A, L109H, K111F, K129S and Y139O. We have also made a mutant of the α4 subunit which forms receptors with β2 partially sensitive to NBT (15.9±1.4% block by 1μM NBT) and has a proline at position 198, as does α2. Changing pro198 of α4, to gln as in α3, increases NBT sensitivity (11.6±6.8% block by 1μM NBT). These results show thr143 to be a major determinant of NBT sensitivity and provide additional evidence for a role of gln198 in determining NBT sensitivity.

**466.36**

**MAPPING THE NEURONAL NICOTINIC RECEPTOR BETA SUBUNIT CONTRIBUTION TO BLOCKADE BY THE COMPETITIVE ANTAGONISTS DHIHYDO-ε-ERYTHROIDINE AND NEURONAL BUNGAROTOXIN S. C. Harvey* and C. W. Luebic. Department of Molecular and Cellular Pharmacology, University of Miami, Miami, FL 33101.

Neuronal nicotinic acetylcholine receptors (nACHR) can be formed in Xenopus oocytes by injecting various combinations of cRNA encoding two classes of homologous subunits, α and β. Each subunit combination has distinct pharmacological properties, with both α and β subunits contributing to ligand sensitivity. We constructed β subunit chimeras to map determinants of sensitivity to two structurally distinct competitive antagonists, dihydro-ε-erythroidine (DHβE) and neuronal bungarotoxin (NBT). Receptors formed by α3 and β2 are sensitive to block by 100 nM NBT (97.2±2% block), while α3β4 is insensitive. At AC concentrations approximating the EC50, α3β2 is 50 fold more sensitive to DHβE (IC50=400 nM) than α3β4 (IC50=20 μM). 3 μM DHβE effectively blocks α3β2 (90±4.6% block) but has little effect on α3β4 (13±5.1% block). Substituting the first 135 N-terminal amino acid residues of α4 into β2, results in a subunit which forms receptors with β4-like ligand sensitivity, i.e. insensitive to both 100 nM NBT and 3 μM DHβE. Substituting the first 105 N-terminal residues had similar results. Substituting in the first 58 residues of β4 resulted in intermediate sensitivity to NBT and DHβE (80±1.7% block by 100 nM NBT; 71±6.8% block by 3 μM DHβE). These results demonstrate that at least two distinct sections of the β subunit (1-58 and 58-105) are involved in determining receptor sensitivity to competitive antagonists.

**467.1**

**SYNTHESIS AND RECEPTOR BINDING OF NOVEL CHOLINERGIC CHANNEL LIGANDS AS POTENTIAL Cognition Enhancers**


Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by a global deterioration of cognitive function. (-)-Nicotine has reported beneficial effects in AD to improve cognitive performance. Preclinical testing (Henderson et al.; Decker et al., J. Pharmacol. Exp. Therap. in press, 1994; Garvey et al., J. Med. Chem., in press, 1994.) has recently demonstrated that ABT-418, L3 (5-methyl-5-(1-methyl-2-pyrrolidinyl)-1(2H)-pyridinone) is a potent binding agonist that has fewer in vivo liabilities than (-)-nicotine. Like (-)-nicotine , however, ABT-418 possesses poor oral bioavailability. In order to assess what structural changes in ABT-418 are consistent with high affinity binding, an extensive SAR has been conducted on L3. The compounds prepared were found to have binding affinity ranging from 2.2-6,000 nM (displacement of [3H]cytisine from whole rat brain synaptic membrane). The SAR results indicated that a bulky alkyl group or function is not well tolerated for the potent binding affinity. We report here the synthesis and receptor binding of a series of pyrrolidinyl modified isoaxazole analogs.

**467.2**


DMXBA-Anabancine has recently been found to enhance learning in aging rabbits (Woodruff-Pak, Li, and Kent, 1994) and rats, as well as in nucleus basalis-lesioned rats (Meyer et al., 1994). Experiments with nicotinic receptor subtypes expressed in oocytes have shown that the compound acts as a very weak partial agonist upon the alpha-4-beta2 combination, but as a strong partial agonist upon homo-oligomeric alpha 7 channels (Papeke et al., 1993). It enhances LTD in the hippocampal slice (Hunter et al., 1994). We have indirectly investigated the interaction of DMXBA with several naturally expressed nicotinic receptors by measuring displacement of nicotinic radioligand binding to brain synaptosomes, modulation of PC12 (p75) neurite outgrowth, and to the neuronal nicotinic alpha7 subunit (Papeke et al., 1993). It enhances LTD in the hippocampal slice (Hunter et al., 1994). We have indirectly investigated the interaction of DMXBA with several naturally expressed nicotinic receptors by measuring displacement of nicotinic radioligand binding to brain synaptosomes, modulation of PC12 neurite outgrowth, and to the neuronal nicotinic alpha7 subunit (Papeke et al., 1993). It enhances LTD in the hippocampal slice (Hunter et al., 1994). We have indirectly investigated the interaction of DMXBA with several naturally expressed nicotinic receptors by measuring displacement of nicotinic radioligand binding to brain synaptosomes, modulation of PC12 neurite outgrowth, and to the neuronal nicotinic alpha7 subunit (Papeke et al., 1993). It enhances LTD in the hippocampal slice (Hunter et al., 1994). We have indirectly investigated the interaction of DMXBA with several naturally expressed nicotinic receptors by measuring displacement of nicotinic radioligand binding to brain synaptosomes, modulation of PC12 neurite outgrowth, and to the neuronal nicotinic alpha7 subunit (Papeke et al., 1993). It enhances LTD in the hippocampal slice (Hunter et al., 1994).
467.3

The effects of nicotinic receptor ligands on performance in a task measuring sustained attention, vigilance, or vigilance. This task required the animals to discriminate between signal and non-signal events. The sequence of signal (central panel light illumination for 500, 50 or 25 msec) and non-signal presentations was randomized over 3 blocks of 54 trials each (27 signal trials, 9 per length, and 27 non-signal trials). A left lever press following a signal was counted as a hit, and a right lever press following a non-signal event was counted as a correct rejection. Hits and correct rejections were rewarded, whereas misses and false alarms (defined as incorrect right and left lever presses, respectively) were not. Baseline performance was characterized by a signal length dependent ability of the animals to discriminate between signal and non-signal events. Administration of nicotine (0.19, 0.62, 1.9 μmol) did not produce main effects on vigilance performance. The putative nicotinic receptor agonist lobeline (1.9, 6.2, 19 μmol) impaired the animals' ability to discriminate between signal and non-signal events. The antagonist mecamylamine (5, 15, 50 μmol) produced impaired vigilance performance. While the detrimental effects of lobeline may have been related to the effects of direct cholinergic stimulation in intact animals, the reasons for the differences between the effects of nicotine and lobeline remain unsettled.

467.4

Epidatidine (EB) is reported to be an equipotent agonist of the nAChR from chicken (Kj=3 μM). The agonist effects were investigated by electrophysiological recordings from perfused chick retinas, and by radioligand assays for various nicotinic receptor subtypes, using [3H]-nicotinic acid and nicotine for competition. A 2-sec application of EB (10 μM) caused a curare-sensitive depolarization, but subsequent depolarizations due to the nicotinic agonist dimethylphenylpiperazinium (DMPP, 300 μM, 2 sec) were blocked by >70%. Depolarizations due to glutamate agonists kainate and NMDA were not affected. EB (10 μM) was the minimum dose to completely desensitize DMPP responses in retina when applied continuously, but DMPF responses recover 50% of nicotine blockade within ~20 min. In contrast, 100 nM EB (20 min) completely blocks DMPF responses, and recovers in 1 hr for recovery. Preliminary data suggests that d-tubocurarine (200 μM) prevents long-term desensitization by EB. EB displaces ['H]-curarine binding to α2β2 receptors by >30% and [3H]-nicotinic acid binding to α7-containing receptors (Kj=22 nM) immunosioaled from chick brain. The corresponding Kj for nicotine or anagonists were 20 nM and 80 nM for α11 and 140 nM or 22 μM for 2H-α-bungarotoxin, respectively. In addition, EB blocked α2β2- and α7-bungarotoxin binding to membranes containing Torpedo α1 receptors with an IC50 of 100 nM. The results indicate that EB is a strongly desensitizing nicotinic agonist that may be a useful ligand for several receptor subtypes. Supported by NIH NS22472.

467.5
COMPARATIVE PHARMACOLOGY OF EPIDATIDINE, A POTENT SELECTIVE AGONIST FOR NEURONAL AChRs.

The pharmacological properties of the (+)- and (-)-isomers of the synthetic analgesic epidatidine (EB), originally isolated from the skin of the frog, Epipedobates tricolor, were tested on different chicken and human neuronal AChRs. In competition binding assays EB was used on immunonaffinity isolated chicken brain (α7,4β7,0), and human neuronal nicotinic (α3 and α7)AChR from the SHSY-5Y cell line. Both EB isomers exhibited extremely high affinity for all neuronal AChRs tested, with IC50 values ranging from 1μM (human α3 AChR) to 1μM (chicken α7 AChR). By contrast, no EB binding was observed on human muscle type AChR from the cell line TE671. EB behaved as an extremely potent full agonist on chicken α3β2, α3δ, αβ2, α7, and α8 and human (α3β2) neuronal AChRs expressed in Xenopus oocytes. Currents induced by EB were effectively blocked by the nicotinic antagonists hexamethonium and mecamylamine. Apparent affinity was 100 to 1000 times higher for EB as compared to nicotine (α6 current values ranged from 1 μM for homoronic chicken α7 to 2μM for homoronic chicken α7). EB did not activate or block expressed Torpedo muscle type AChRs at concentrations up to 1μM. Currents induced by EB in oocytes expressing chicken αβ2 and α3β2 showed significantly slower desensitization and inactivation kinetics than did currents induced by ACh and nicotine.

467.6
FURTHER CHARACTERIZATION OF THE IN VIVO EFFECTS OF (±)EPIDATIDINE, A POTENT NICOTINIC LIGAND.

Epidatidine has been reported to be a potent nicotinic ligand that can produce analgesia (Qin et al., Eur. J. Pharmacol., 250, 1993; Bado and Daly, Mol. Pharmacol., in press). This study further characterized epidatidine-induced analgesia, and examined additional in vivo effects of epidatidine. Consistent with earlier reports, (±)epidatidine (0.1 μg/kg, i.p.) had antinociceptive activity in mice (±) epidatidine, producing an analgesic effect in the hot-plate paradigm. The analgesic effect of epidatidine was evident at 60 min post-injection (p < 0.05) but not at 120 min (p < 0.05). Although mecamylamine (1.5, 5, and 15 μg/kg, i.p.) pretreatment attenuated analgesia, mecamylamine (15 μg/kg, i.p.) administered after epidatidine did not attenuate the analgesic response. At the analgesic dose of epidatidine (0.1 μg/kg) utilized in this study, significant reductions in activity and body temperature were observed. Since these effects (e.g., motor effects) possibly contributed to the apparent analgesic effect of epidatidine, additional studies were conducted with mice pretreated (i.p., 5 days prior to analgesia testing) with the nicotinic antagonist chlorisondamine (23 μg/kg, i.p.). In mice pretreated with chlorisondamine, the analgesic effect of epidatidine was attenuated, but there were virtually no effects on the epidatidine-induced decreases in activity or temperature. These data suggest that epidatidine produces analgesia by a central (or spinal) mechanism that can be differentiated, in part, from effects on temperature and activity.

467.7
FURTHER STUDIES ON THE ROLE OF CENTRAL NICOTINIC RECEPTORS IN THE ANALGESIC EFFECTS OF EPIDATIDINE.
B. Badia*, H.M. Garaffo, D. Shi and J.W. Daly* Laboratory of Biocorganic Chemistry, NIDDK/NHL, Bethesda, MD 20892.

Epidatidine is a potent analgetic agent, whose site of action appears to involve central nicotinic receptors. However, epidatidine elicits analgesia even in a mouse strain in which nicotinic activity has been selectively blocked by 'H'nicotine binding. In addition, epidatidine is not tolerant to the behavioral effects of nicotine by chronic nicotine or chronic caffeine, the analgesic effects of epidatidine are reduced. Epidatidine is about 20-fold more potent than nicotine at rat brain nicotinic receptors, about 200-fold more potent at ganglionc-type receptors and about 100-fold more potent at muscle-type receptors. Epidatidine blocked responses to epibatidine blocked like those of nicotine by both competitive and noncompetitive nicotinic antagonists. Epidatidine, like nicotine, causes desensitization of nicotinic receptors. Unlike nicotine, there is little enantioselectivity in the responses of epidatidine, either in vivo, in binding and functional assays with brain preparations, or in cultured expressing ganglionic or muscle-type nicotinic receptors. Furthermore, unlike the case for nicotine/nornicotine the presence or absence of an N-methyl group has little effect on activity of epidatidines. Molecular modeling provides a rationale for the effects of N-methylation in the nicotine and the lack of such effects in the epidatidines.

467.8
ENANTIOMERS OF EPIDATIDINE AS POTENT NICOTINIC AGONISTS AT TWO IDENTIFIED SUBTYPES OF NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs) IN RAT HIPPOCAMPAL NEURONS. M. Alkondon*, T. Bannon and F.X. Albuquerque. These data were presented at the 1994 Society for Neuroscience, Abstracts.

Epidatidine, a novel analgesic compound isolated from the Ecuadorian frog Epipedobates tricolor, is a potent analgesic in mice (Spande et al., J. Am. Chem. Soc. 114: 3475, 1992). Recent reports (Klein et al., J. Pharmacol. Exp. Ther. 1994) have also shown activity against the isolation of epidatidine from E. tricolor. The enantiomers of epidatidine are more potent than the (+)-enantiomer and have enantiomers that are 2 to 5 μM. The enantiomers of epidatidine were activated by epidatidine in the low nanomolar range (apparent EC50 between 0.2 and 10 nM). The natural (+)-enantiomer is being tested in the rat. The results indicate that epidatidine enantiomers can distinguish between the subtypes of nAChRs present in the hippocampal neurons as 1) they are about 1000-fold more potent in evoking the type I enantiomer than the type I enantiomer and 2) the order of potency of the enantiomers of epidatidine was reversed for the two types of currents. The concentration of epidatidine necessary to activate the type I current is compatible with the doses that evoke an analgesic effect in mice, and suggests the involvement of the α4β2 nACh subtype in such effect. Support: NINDS Grant NS25296, NIH/NIGMS Grants E50730 and E507263.

(―)Anatoxin-a (ANTX) stimulates guinea pig ileum contraction with a potency similar to acetylcholine (ACh), and this is due to the induced release of ACh from the ileum. The postulated target of ANTX is the nicotinic receptor on ganglionic interneurons of ileum, as evidenced by the inhibition of ANTX-induced contraction by muscarinic or nicotinic antagonists. When tested on the ANTX-stimulated guinea pig ileum, it was found that adenosine receptor agonists, N-cytisine, adenosine (CHA), S-N-ethylcarboxamido-adenosine (NECA), and N6-phenylisopropyl-adenosine (PIA), all blocked the contraction with CHA being the most potent. These findings suggest that the ganglionic nicotinic receptors are under complex regulation, with possible modulation either directly or indirectly with A2 subtype adenosine receptors.


The local anesthetic bupivacaine has been reported to be an open channel blocker of nicotinic receptors (nAChRs) in cultured rat hippocampal neurons (Abel, Soc. Neurosci. 19:1535, 1993) and in frog motor neurons (Med. Pharmacol. 26:293, 1984). In the present study, we further investigated the interaction of bupivacaine with hippocampal a402 receptors using patch-clamp techniques. Whole-cell currents were recorded from rat hippocampal neurons cultured for 10-30 days. Bupivacaine was applied by perfusion in the bath or in pulses together with ACh via a U-tube. The peak amplitude of the hippocampal ML-endocannabinoid currents, produced by ACh and decreased by bupivacaine, could be reduced by bupivacaine in a concentration-dependent manner. In addition, when the neurons were incubated with 50 μM bupivacaine prior to activation of a402 receptors by 400 μM ACh, the blockade increased with Downcurrent time (2-8 min). This time-dependent blockade is suggestive of a binding site for bupivacaine distinct from the site within the open channel that has been identified previously in neuronal and muscle nAChRs. The blockade of bupivacaine was also evidenced by a decrease in peak amplitude of the fast decaying currents without a significant change in the decay time constants. The lack of voltage dependence of bupivacaine blockade of the fast decaying currents also indicated that the binding sites for bupivacaine on nAChRs is outside of the voltage-sensitive region of the channel itself. Competition experiments showed that the 18-20% blockade of fast decaying nicotinic currents by 100 μM bupivacaine remained unchanged over a wide range of concentrations of ACh (100 μM to 10 nM), indicating that the binding by bupivacaine to this type of receptor was noncompetitive and was not allosterically affected by the agonist. The action of bupivacaine on the closed channel, observed on neuronal nAChRs, was not reported for muscle nAChRs. (Support: USPHS Grant NS 23296)

EFFECTS OF STEROID EXPOSURE ON LIGAND BINDING AND FUNCTIONAL ACTIVITY OF NICOTINIC ACETYLCHOLINE RECEPTORS. LeL. E* and Ronald J. Lukas. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013, USA.

To explore potential mechanisms for the differential modulation of nicotinic acetylcholine receptor (nAChR) expression and function during development and in response to environmental challenges, we tested how exposure to steroids, such as progesterone, estradiol, testosterone, corticosterone and dexamethasone, affects function and numbers of diverse nAChR subtypes. Acute steroid exposure has no effect on [3H]acetylcholine and/or [125I]-labeled α-bungarotoxin (Bgt) binding to human muscle-type nAChR (of the T671/RD clonal line) or on radioligand binding to human ganglia-type nAChR containing α3 and β2 subunits or to human nicotinic Bgt binding sites containing α7 subunits (of the SH-SY5Y neuroblastoma). However, steroid exposure inhibits function (assessed using [3H]nicotine) of both muscle- and ganglia-type nAChR non-competitively with IC50 values in the low-intermediate μM range, depending on the specific steroid studied, with progesterone displaying highest nAChR affinity. Assays done using steroids conjugated to abainus indicate that these functional effects are due to steroids interacting with extracellular domains of nAChR. These studies suggest that fluctuation in local steroid levels could influence nAChR function acutely, via apparently allosteric mechanisms.
TOXICITY OF NICOTINE FOLLOWING CHRONIC TREATMENT: A POTENTIAL ROLE FOR CALCIUM CHANNELS. M.I. Damaj & B. Martin, Department of Pharmacology and Toxicology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23220, USA.

Recent findings suggest a role for the L-type calcium channels in the pharmacological effects of nicotine. The purpose of this study was to determine whether calcium channels were involved in nicotine behavioral tolerance in mice. Male ICR mice were chronically administered nicotine (5 mg/kg, i.p.) and nicotine (2 mg/kg, s.c.) twice a day for 10 days. At day 11, mice were challenged with different doses of nicotine and the effect on the tail flick test was measured. In another group of mice, nicotine (2 mg/kg, s.c.) was given chronically to mice for 10 days and the acute nicotine challenge was performed 21 days after the last injection. At day 11, mice were challenged with different doses of BAYK8644 and its effect on motor coordination (rotated test) and locomotor activity was measured after i.p. administration. The effect on the tail flick test was assessed after intrathecal (i.t.) administration of BAYK8644. Chronic co-administration of nimodipine with nicotine, significantly reduced tolerance to the antinociceptive effects of nicotine. On the other hand, BAYK8644 effects in nicotine-treated mice decreased. Indeed, the ED$_50$ for BAYK8644-induced motor impairment increased from 0.35 to 2.0 mg/kg in tolerant mice. In addition, the ED$_50$ for BAYK8644-induced antinociception after i.t. injection increased from 3.7 g/mouse to 12 g/mouse. These results suggest that L-type calcium channels play a role in nicotine tolerance in mice, and further experiments in mice are needed to explain the mechanisms underlying these effects. (Supported by NIDA grant #DA-05274).

ACETYLCHOLINE RECEPTORS: NICOTINIC PHARMACOLOGY

CALCINEURIN AND PKC REGULATE ACeR FUNCTION AT SNAKE TWITCH FIBER ENDPLATES. L. Pandol & J.C. Hardwick, Department of Anatomy & Neurobiology, University of Vermont, Burlington VT 05405, USA.

Staurosporine decreases the extent of recovery of endplate sensitivity following prolonged exposure to calcium, presumably by inhibition of protein kinase activity (Hardwick & Pandol, Br J Pharmacol 108: 741, 1991). Experiments have been done to determine which protein kinase is responsible for full recovery from agonist exposure. All experiments were performed in vitro using two populations of sensory motor endplates, one containing muscles maintained in an isotonic potassium propionate solution. Following treatment with 50 μM spingosine, an inhibitor of CAMK and PKC, the extent of MEPC amplitude recovery following 40 min exposure to 540 μM nicotine was decreased by 30%. Conversely, KN-62 and lavendustin A, inhibitors of CAMK II and tyrosine kinase respectively, had no effect on MEPC amplitude recovery. Chronic treatment (18-20 h) with 200 μM PMA, which down regulates PKC, also reduced the extent of MEPC recovery by 27%. In contrast, the inactive analog aPMA had no effect. Following calcium exposure in PMA-inhibited preparations, two populations of ACeR activated channels, one of pS and one of pN, were observed. In untreated preparations, only the large conductance channel was observed. Treatment of preparations with 0.5 μM daniethisine, an inhibitor of protein phosphate 2B (kinase), prevented the decrease in MEPC recovery associated with PKC inhibition and decreased the occurrence of small conductance channels. These observations suggest that ACeR's or associated proteins are dephosphorylated by calcium during prolonged agonist exposure.

Further results in a population of ACeR channels with a reduced conductance. Full recovery of these dephosphorylated ACeR complexes requires rephosphorylation by PKC. Supported by NIH grant NS 23978.


Vasoactive intestinal polypeptide (VIP) and substance P (SP)-immunoreactive neurons and nerve fibers have been shown to be present in the mammalian intracerebral ganglia (Weibe et al., 1984, Cell Tiss Res. 236, 527). The effects of VIP and SP on ACeR evoked currents were investigated in cultured rat parasympathetic cardiac neurons under voltage-clamp, using standard whole-cell, perforated-patch, and outside-out recording configurations of the patch clamp technique. Pressure ejection of VIP onto the soma increased the ACeR-evoked current amplitude ~2-fold, with half-maximal potentiation occurring at 260 μM VIP (τ = 3). VIP also potentiated the current evoked by nicotine (100 μM), but not those evoked by muscarine or ATP. Mecamylamine (3 μM) completely inhibited the currents elicited by ACeR and nicotine in the presence of VIP. Post-inoculation of cultured neurons with neurotransmitters (e.g., VIP, SP, 30-100 ng/ml), intracellular application of GDF-DPS (100 μM) and bath application of L-88 all blocked VIP-inhibited potentiation, suggesting that the effect of VIP is mediated by a G-protein coupled receptor. In outside-out patches, bath-application of ACeR (4 μM) and VIP (4 μM) reduced the mean closed time between bursts of ACeR receptor-channel activity by 36% (n = 3) compared to that observed with ACeR alone. The mean closed time between bursts of evoked currents was reduced by VIP, suggesting that VIP may increase the rate of desensitization of the ACeR receptor-channel activity. Since ACeR is the primary neurotransmitter of extrinsic (vagal) innervation of the mammalian heart, VIP and SP may play an important role in modulating autonomic control of the heart.


It has been demonstrated that (-)ephedrine, certain polyanilines, and 4-methylpyrrolidines (4-MP) can activate nicotinic receptors (nAChRs) expressed in Torpedo electric organ. Here, we investigated the possibility that binding to a nAChR site distinct from that for acetylcholine (ACh). This novel pathway of nAChR activation was shown to be insensitive to competitive nicotinic antagonists and to nAChR desensitization. High concentrations of ACh were shown to be sensitive to the blockade by the nAChR-specific monoclonal antibody FK1. In this study, we evaluated whether agents known to enhance ACh-induced muscle nAChR desensitization (e.g. pentoxyfylline) or to produce blockade of muscle nAChR in its open conformation (e.g. atropine) also affect the 4-MP-induced nAChR activity in L. suoliaris muscle fibers under cell-attached condition. Whereas pentoxyfylline (61.1 μM) reduced 4-MP-induced nAChR desensitization, 7 μM atropine did not. ACh- and 4-MP-induced single-channel activity. Short-lived isolated single channels and no evidence for burst activity were observed, indicating a rather slow rate of unblocking for atropine. Our results indicate that the desensitization by non-competitive blockers, similar to that induced by high concentrations of ACh, upregulates the ACh-binding site from channel gating, and does not prevent nAChR activation via another pathway. On the other hand, nAChR channels opened via either pathway are accessible to the action of an open-channel blocker. Support: Molec. Pharmacol. Train. Prop. FINERP/UMAB, FINERP and CNPq.

RE-APPRaising THE BUNGAROOTOXIN-Sensitivity OF NICOTINIC RECEPTORS ON BULLFROG SYMPAThETIC NEURONS. J.P. Horn*, W.K. Shen and P Kohling, Department of Neurobiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

An earlier study reported that α-bungarotoxin (α-bgt) blocks synaptic transmission in bullfrog sympathetic ganglia (Marshall, PNAS, 78, 1948,1981). This unusual finding suggests that nicotinic receptors on amphibian autonomic neurons may be fundamentally different from neuronal nicotinic receptors in other ganglia (see Libschome and Rang, J. Neurosci 8, 3258, 1988).

We have studied the sensitivity of nicotinic synapses to α-bgt and neuronal-bungarotoxin (n-bgt) in the B and C cell systems of bullfrog sympathetic ganglia 9 & 10 by recording compound postganglionic action potentials from rami communicantes. High concentrations (10 μM) of α-bgt applied for up to 8 hours had no effect upon synaptic transmission in either the B or C cell system. Ganglia pretreated with carbachol were also insensitive to α-bgt. In contrast, n-bgt concentrations on isolated sartorius muscle preparations, nerve-evoked twitches were fully blocked by 30-100 nM α-bgt. By contrast, 30-300 nM n-bgt blocked nicotinic transmission in the B and C cell system, one block was observed within 25-45 min and reversed fully with a half-time of 40-80 min. This was indistinguishable from washout times after block by 100 μM d- tubocurarine. Based on their sensitivity to n-bgt, the B and C neurons could be classified as neuronal in type. Supported by a Grant-in-Aid and a Postdoctoral Fellowship (PJ) from the American Heart Association, PA Affiliate and NIH grant NS01427.
ACETYLCHOLINE RECEPTORS: NICOTINIC PHARMACOLOGY

A COMPARISON OF NICOTINIC RECEPTORS ON SYMPATHETIC B AND C NEURONS: KINETICS, VOLTAGE-DEPENDENCE AND COMPETITIVE BLOCK BY D-TUBOCURARINE W-X Shen and H-P Horn. Dep't of Pharmacology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Previous studies have shown that nicotinic receptors on sympathetic B and C neurons differ in their kinetics (Shen & Horn). Nicotinic receptors in B and C neurons of the rat (Shen & Horn, Neurosci. Lett. 595/356/1993) and that synaptic transmission in the C system is more sensitive to d-tubocurarine than in the B system (Shen & Horn, Neuroscience 595/356/1993). We have now used two-electrode voltage clamp recordings to analyze the voltage-dependence of receptor kinetics and DTC's actions on these two cell types. DTC's effect on the capacity factor (r) that is shorter in B than C cells (5.1±0.6 vs 8.9±1.2 ms at -50 mV). EPSC's in both cell types have similar reversal potentials (-3.9 vs -5.6 mV) and show little rectification at negative potentials. The voltage-dependence of r is also similar in B (0.003±0.001 ms/V, n=28) and C (0.003±0.001 ms/V, n=13) cells. In both cell types, 3 mC/μm2 DTC reduces EPSC amplitude to the same extent, independent of membrane potential (-50 mV): 41% in B cells, n=8 and 47% in C cells, n=4, P<0.05; -100 mV: 47% in B cells, n=6, and 50% in C cells, n=3, P<0.05). DTC has no effect upon the shape of the I-V relation or r. These results provide evidence that nicotinic receptors on B and C cells are similar in every way except for r. In both cell types DTC acts as a competitive antagonist with similar affinity. Transmission in the C system is more sensitive to DTC because of its lower synaptic conductance (B:199±nS, C:154±nS). Supported by a Grant-in-Aid from the American Heart Assoc., PA Affiliate and NIH NS21605 & NS01427.


Previously, we demonstrated that the concentration of the cholinesterase (ChE) inhibitor Alicardin is increased, there is a potentiation and then a depression of nerve-elicited muscle twitch, and that these effects cannot be fully accounted for by ChE inhibition. In this study, we have examined the effect of one single dose of Alicardin (100-800 nM) on the activities of nAChRs in muscle fibers from rats 3 hours after a single dose of Alicardin (100-800 nM) was given. Alicardin increased the peak amplitude of ACh-elicited currents (ACh) and reduced the amplitude of the fast-decaying component. Alicardin did not affect the amplitude of the slow-decaying component. These results suggest that the effects of Alicardin on the ACh-elicited currents may be due to an increase in the number of active AChRs in the muscle fibers. This is consistent with the findings of previous studies that have shown that Alicardin increases the activity of cholinesterase in muscle fibers. Therefore, the increased activity of AChRs in muscle fibers may be due to an increase in the number of active AChRs in the muscle fibers.


Our previous study on cultured hippocampal neurons revealed the presence of four distinct types of currents, of which, one type, 1A, was the predominant response (JPET 245: 1995). To determine the most common type of nicotinic currents in neurons that have developed in vivo, we performed single channel experiments and found that the neurons were activated by nAChRs. We conclude that the hippocampal neurons are activated by nAChRs and that the current was due to an increase in the number of active nAChRs in the neurons. The results suggest that the current is due to a decrease in the number of active nAChRs in the neurons.
ACETYLCHOLINE RECEPTORS: NICOTINIC FUNCTION

468.1

RECEPTION OF ALPHA-BUNGAROTOXIN-SENSITIVE, NICOTINIC WHOLE-CELL CURRENTS EVOKED IN RAT BRAIN NEURONS IS Mg²⁺-DEPENDENT: DEFENSIVE RESPONSES OF CULTURED NEURONS TO NEUROTRANSMITTERS. 
Exper. Eye Res. 64: 159-169, 1997.)

468.2

ELECTROPHYSIOLOGICAL EVIDENCE FOR PRE- AND POSTSYNAPTIC NICOTINIC RECEPTORS IN THE RAT INTERMEDIATE CELL COLUMN. 
Nucleus A. Bordery, M. Schmitz, M. Leibing, M. H. Aminzadeh, M. G. H. 

We studied the responses of cholinergic nicotinic agonists, DMPM (Dimethyl-4-phenyl-piperazinum iodide) and nicotine in thin (200 µm) transverse slices of the thoracic spinal cord from rats (NIH: CD-1, 35-60 g), using the whole-cell recording configuration of the patch-clamp technique. Single rat hippocampal neurons (NRIPs) and newborn rat sympathetic neurons (SPNs) on the basis of their localization (intermedinorad cell column (IML)), morphology and electrophysiological properties (resting potential 48.61±0.14 mV; membrane resistance 1,650±255 MΩ; presence and of an A ChR). In SPNs, DMPM and nicotine had two effects: 1a) shifting current-voltage relationship that desensitization (n=12/21) and 2) long term effect which is the induction of fast oxytocin and/or inhibitory currents (respectively EPSCs and IPSCs) lasting 20-30min after the end of the nicotine brief application. IPSCs were desensitized by nAChR-IR application either by iontophoresis or by pressure induced an inward current accompanied by an increase in resting membrane potential. A first transient peak (-181.3±10.4%, n=3) showing a fast desensitization followed by a plateau phase (-78.8±14.65%, n=7). This second phase diminished in amplitude after successive applications of DMPM (reduction between two successive applications of DMPM (2sec) spaced by 3min is 38.0±8.0%, n=6). It reversed at 0mV and was not abolished by perfusion of lanthanum (200µM). As regards the long lasting effect, each nicotinic agonists (10-20sec) triggered the appearance of EPSCs and/or IPSCs in SPNs and in intermedinorad neurones. These results suggest that synaptic receptors are located postsynaptically on SPNs and preysynaptically on both glycinate and intermedinorad neurones forming triadic terminals that project either to SPNs or to unidentified neurones of the IML.

468.3

CHARACTERISTICS OF NICOTINIC WHOLE-CELL CURRENTS IN P12 CELLS. 

The 9-acetate (nAChR) that gives rise to each of these three types (IA, II, and III) of acetylcholine currents evoked in a-cleft hippocampal neurones is identified on the basis of the pharmacological and kinetic properties of the currents. A 9-acetate (nAChR) has been assigned to type IA current, an 9-acetate (nAChR) to type II, and an 9-acetate (nAChR) to type III (Peitler, 1969). Although the cloned nAChRs of the bovine retina (PC12 cells) represent an established model for the study of synaptic ganglionic transmission, and expression of functional nAChRs in the PC12 cell line has been demonstrated (Storch, 1991), these results suggest that the acetylcholine currents evoked in these cells have not been assigned to specific nAChR subtypes. In the present study, the characteristics of nicotinic whole-cell currents activated in cultured sympathetic neurons were compared with those of a-cleft hippocampal neurones. Application of ACh (3 µM) via a U-tube system to PC12 cells evoked whole-cell currents whose peak amplitudes increased with ACh concentration; the EC50 for ACh was ≈ 75 µM. Whereas nicotinic currents in PC12 cells were insensitive to d-tubocurarine (100 µM), they could be inhibited by methyllycaconitine (MLA, 1 µM), in this way resembling nicotinic currents in a-cleft hippocampal neurones. In addition, a long-lasting blockade was observed when MLA was applied simultaneously via the bath perfusion and the U-tube, and ACh-evoked currents in PC12 cells decreased with a slow time constant (τ) for the ACHE-mediated currents of ≈ 0.250 ms in PC12 cells. Thus, whereas the sensitivity of ACh-evoked currents in PC12 cells to competitive antagonists blocked those of the PC12/AChR-evoked type IA current in hippocampal neurones, the decay and the MLA-induced long-lasting blockade of nicotinic currents activated in the PC12 cells were different from those of type IA currents. It is possible that a neuronal nAChR made up of a combination of a subunit(s) and other neuronal nAChR subunit(s) subserves the nicotinic currents in the PC12 cells. Support: USPHS grants NS 25296 and NS 05730.

468.5

EFFECTS OF DENERVATION UPON ACETYLCHOLINE RECEPTOR CLUSTERS IN AUTONOMIC NEURONS AS DETERMINED BY QUANTITATIVE LASER SCANNING CONFOCAL MICROSCOPY. 

We previously showed that denervation altered properties of acetylcholine receptor (AChR) clusters on the surface of frog cardiac ganglion cells (Neuron 1: 877-886, 1988). We have extended this analysis by using a laser scanning confocal microscope to optically section and reconstruct neuronal clusters of AChR clusters that are spatially co-localized with sympathetic neurons. AChR clusters were labeled with antibodies to the rat cardiac AChR α2 subunit, which is a specific marker for sympathetic neurons. AChR clusters were identified using a laser scanning confocal system (Olympus). On normally innervated ganglia, AChR clusters were co-localized with sympathetic boutons and were spatially clustered on the cell surface, since soluble factors released from boutons which are themselves clustered. On denervated neurons, by contrast, AChR clusters are spatially dispersed. Denervation also leads to a reduction in the relative number of AChR clusters attributable to enhancement of the extracellular space. However, the extracellular AChR was measured specifically, it was not found to be altered significantly by denervation. Quantitative histochemical studies further showed no change in the extracellular solution. The reduction in the number of acetylcholine receptors (AChRs) that are available for binding ACh does not appear to alter the distribution of AChRs relative to AChE, thus making it unlikely that supersensitivity to ACh results from the migration of AChRs to areas of the cell membrane containing relatively little AChE. (Supported by NIH Grant NS 42072.)
COGNITIVE EFFECTS OF NICOTINIC-CHOLINERGIC MANIPULATION ON A RAT SYMPATHETIC-GANGLION DISCRIMINATION TASK. 


The purpose of this study was to determine the nature of cognitive enhancement following central nicotinic receptor stimulation in a novel version of the delayed nonmatch-to-sample discrimination task. Under this paradigm, an animal is presented with a stimulus, which is followed by a variable length delay (retention) interval, at the end of which the animal has the opportunity to respond, and then there is a true test. In our experiment, a light (visual) stimulus was utilized. To dim the rat's ability to use position and spatial strategies to solve the task, we used computer automated computerized model which separates the animal from the levers during the delay interval, thus preventing positioning at the lever. After stable baseline were reached, animals received or lobeline mesorized as a randomized dose series. Each rat received two complete series of the two drugs on different occasions. Only 2 hours per dose were administered. Optimal doses of both nicotine and lobeline significantly improved overall performance in the delayed stimulus discrimination paradigm by 12.5 and 8.4%, respectively. A differential pattern of improvement was observed when the data was analyzed by delay, with the greatest enhancement being at the longest delays (16.5%) whereas the most significant enhancement with lobeline occurred at the shortest delays (13.7%). In addition, mocainyl 1 mg/kg (a non-nicotinic agonist) administered only at the 30% of the dose series. Enhanced the data, but did not significantly reduce the enhancement caused by lobeline. These data suggest that lobeline's cognitive effects may be expressed through non-nicotinic mechanisms or involve a nicotinic receptor which is not responsive to mocainyl.
469.3 CHARACTERIZATION OF 3H DICHLOROQUINUCREIC ACID BINDING IN CLONED NMDA RECEPTORS EXPRESSED IN TRANSFECTED CELLS. N.J. Angegna, D.R. Lyon, B. S. Sapper, T. D. Brown, J. H. Morris, D. J. Fink. Departments of Pharmacology & Neurology, University of Pennsylvania; Children's Hospital of Philadelphia, PA 19104

The NMDA subtype of receptors for excitatory amino acids contains a glycine recognition domain. Occupation of the glycine recognition site is required for receptor activation. Multiple NMDA receptor (NR) subtypes have been recently cloned. We have transiently expressed NR1 (a and g) and NR2 A-C in HEK293 cells singly or in combination. Using 293 cells expressing NMDA receptor subunits, we characterized the pharmacological properties of 3H-Dichloroquinucreic Acid (3H-DCK), a proposed antagonist of the glycine recognition site. Cells transfected with a combination of NR1.a/g and NR2A-C had 3H-DCK binding with a non-specific component at a low affinity. In contrast, we did not observe this in NR1.a/g. Variations between transfected cells with dextromethorphan, an antitussive used primarily in treating pertussis, and NMDA receptors with 3H-DCK binding sites and studied their analogs distribution in rat hippocampus. 3H-DCK binding sites distribution in rat hippocampus. 3H-DCK binding sites distribution in rat hippocampus. 3H-DCK binding sites distribution in rat hippocampus.
469.9

REQUIREMENT FOR THE ACTIVATION OF NMDA RECEPTORS AND POSITIVE ALLOSTERIC INTERACTIONS OF GLUTAMATE, GLYCINE AND POLYAMINES J.C. Marchiolii and M. Baudry. Neuroscience Program, University of Southern California, Los Angeles, CA 90009-2500.

Activation of NMDA receptors by glutamate, glycine and spermine was studied using non-equilibrium [3H]dizocilpine binding to assess increases in the association kinetic of this channel blocker. Glutamate and glycine mutually increased their affinity and affinity to dizocilpine [3H]dizocilpine binding, which were further increased by spermine. Glutamate and spermine appeared initially to be sufficient to enhance [3H]dizocilpine binding, with no need for glycine. However, in these conditions the binding was inhibited by the glycine antagonists 7-chlorokynurenate and DNXQ. Enhancement curves by glycine in the presence of increasing concentration of 7-chlorokynurenate and saturating concentrations of glutamate and spermine indicated that 1) glycine is still required in the presence of glutamate and spermine, 2) spermine markedly increases the affinity for glycine, and 3) a small glycine contamination (0.05% was enough to stimulate [3H]dizocilpine binding in these conditions, thus explaining our initial observations. The increases in glycine affinity produced by glutamate and spermine were additive.

Spermine, by a biphasic effect on [3H]dizocilpine binding, with a stimulatory phase followed by an inhibition at higher concentrations. The potency of spermine for both phases was increased by glutamate, but not by glycine.

These observations indicate that there are positive cooperative interactions between the glutamate, the glycine and the stimulatory and inhibitory polyamine sites of the NMDA receptor, and that glutamate and glycine, but not spermine, are required to activate the NMDA receptor.

469.11


The limited specificity and affinity of available ligands for the NMDA receptor-associated polyamine site has limited investigations of the role of this site in modulating NMDA receptor function. With the goal of identifying more potent and specific compounds we evaluated the effects of several rigid analogues of aracaine, the most potent competitive antagonist of the polyamine site currently available, using [3H]dizocilpine ([3H]MK801) binding to rat brain membranes. An examination of short chain bisguanidines suggested that a chain length of 4-5 was optimal for activity while 2- and 3-carbon aracaine analogues were less potent. Conversion of the guanidines to imidazole diminished activity by approximately 10-fold. Restriction of the flexibility of the chain also proved helpful. The compound shown had an affinity of 0.7μM. Replacement of the benzene ring by cyclohexane decreased the potency by about 10-fold, while substituting an acetylene into the structure of aracaine also decreased the affinity. However, all of these compounds appeared to act at the polyamine site because they were less potent when spermidine was added to the [3H]dizocilpine binding assay.

These studies have identified a novel, potent polyamine site antagonist that may represent a useful lead for compounds to evaluate the functional activity of this compound are currently in progress. Supported by NIH grant DA07409 and ONR grant 413Y001.

469.10

ACTIVATION-LINKED AND ACTIVATION-INDEPENDENT EFFECTS OF POLYPYRIMIDINES ON PCP RECEPTOR BINDING WITHIN THE NMDA RECEPTOR COMPLEX. S.B. Zuk*, D.C. Javitt and M.J. Frangiacco. Depts. of Psychiatry and Neuroscience and Bronx Psychiatric Center, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY 10461.

The phencyclidine (PCP) receptor is located within the N-methyl-D-aspartate (NMDA) receptor-gated ion channel. The functional state of the NMDA receptor complex thus influences parameters of radioligand binding to the PCP receptor, and PCP receptor ligands can serve as in vitro probes for elucidation of NMDA receptor activation mechanisms. PCP receptor binding is stimulated by NMDA receptor agonists such as L-glutamate and by distinct classes of modulatory agents such as glycine-like amino acids and polyamines such as spermine. The present study utilizes a kinetic approach permitting differentiation of PCP receptor binding within closed and activated conformations of the NMDA receptor complex. The results demonstrate that spermine increases radioligand binding to the PCP receptor through two distinct mechanisms. First, spermine, like glycine, increases the probability of time that NMDA channels remain in the open state in presence of L-glutamate, consistent with a role as a positive allosteric modulator of NMDA receptor activation. Second, unlike glycine, spermine increases the affinity of the PCP receptor for its ligands. The latter effect does not appear to reflect increased NMDA receptor activation. Spermine does not induce glycine-like alteration of the EC50 value for stimulation of PCP receptor binding by L-glutamate, suggesting that the effects of spermine cannot be attributed solely to augmentation of glycine binding. These findings demonstrate first, that total specific PCP receptor binding cannot, of itself, be used as an index of NMDA receptor activation and second, that glycine and polyamines differ in the mechanisms by which they potentiate PCP receptor binding. Supported by PHS RDI DA03383 and Dept. of Psychiatry. AECOM T.B. Karasu, M.D., Chairman.

469.12


As a part of our ongoing investigations into drug interactions at the polyamine site we have developed a radioligand binding to rat brain membranes and NMDA-stimulated [Ca2+]i increases and whole cell currents in cultured forebrain neurons. BT4 inhibited [3H]dizocilpine binding with an IC50 of 2.7μM. The effect of BT4 was reduced by spermine in an apparently competitive fashion, consistent with a shift in the agonist dissociation constant. The IC50 for reduction of BT4 binding by spermine was about 0.1μM, about 10 fold more potent than the spermine equivalent. However, these longer BT compounds were insensitive to alterations in the spermine concentration indicating a situation of action other than the polyamine site. Lower modifications of the BT10 structure decreased activity in this assay. BT8, BT10 and BT12 were also potential inhibitors of NMDA-induced increases in [Ca2+]i, with IC50 values of 1.0, 1.5 and 1.0μM, while BT4 was much less potent (0.5μM). BT10 (10μM) had no effect on responses produced by kainate (100μM), AMPA (50μM) or KCl (50mM). The action of BT10 was sensitive to memantine, although we found no evidence for use-dependence. Thus, bisthioureones are a novel class of NMDA receptor ligands that exert actions on one more site on the receptor depending on the size of the molecule.
470.1 EXTRACTS FROM CYPERUS ARTICULATUS (CYPERACEAE)

EXCITATORY NMDA OPENINGS, CONCENTRATION-DEPENDENT CONFERS NITRENIDERINE-RELATED MEDIA TED RESPONSES IN THE RAT CORTICAL WEDGE. E. Ngo Bum, C. L. Mei*, S. Urayvel, F. Wang and P. H. Herrling. Sanford Research Institute CH-3001 Bern and Sanford Research Ltd., CH-4002 Basle, SWITZERLAND.

The marshland plant Cyperus articulatus (Cyperaceae) is commonly used in traditional medicine in Africa and Latin America, to treat a wide variety of human diseases ranging from headache to epilepsy. We tested the hypothesis that the reported anti-epileptic effect of this plant might be due to functional inhibition of the N-methyl-D-aspartate (NMDA) receptor complex. One or several component(s) contained in the extracts inhibited the binding of NMDA to the NMDA recognition site of the NMDA receptor complex from rat neocortex. Water extracts from rhizomes of Cyperus articulatus dose-dependently reduced spontaneous epileptiform discharges and NMDA-induced depolarizations in the rat cortical wedge preparation by a NMDA receptor-mediated mechanism. We conclude that the purported anti-epileptic effect of Cyperus articulatus might at least partially be due to inhibition of NMDA receptor-mediated neurotransmission.

470.2 GLUTAMATE AND GLUTAMINE METABOLITES ON THE ACTIVATION OF N-METHYL-D-ASPARTATE RECEPTORS IN NEURONAL EXTRACTS.

GLUTAMATE INHIBITION BY ETHANOL, J.L. Morris and S.W. Lees*. Div. of Pharmacology/Toxicol., Coll. of Pharmacy, Univ. of Texas, Austin, Texas 78712-1074.

Previous reports have shown that NMDA-stimulated currents in Purkinje cells of the rat cerebellum were reduced by ethanol. At a concentration of 3×10^(-5)M, 10 mM glutamate (Glu) inhibited the NMDA-evoked response by 30%. Glu inhibited NMDA-stimulated currents in a concentration-dependent manner. In addition, NMDA-evoked currents were not inhibited by an equimolar concentration of the NMDA antagonist, 2-amino-5-phosphonopentanoic acid (AP5). These results suggest that ethanol inhibits NMDA receptor-mediated responses in the cerebellum.

470.3 ETHANOL INHIBITION OF HETEROCLUS NMDA CHANNELS IS NOT POTENTIATED BY THE PRESENCE OF glycine.

REDUCTION/OXIDATION OF THE REDOX MODULATORY SITE BY E. Chao, V. Jaturavat* and S. N. Treimann. Department of Pharmacology and Program in Neuroscience, University of Massachusetts Medical Center, Worcester, MA 01655.

Previous reports indicate that the presence of Mg2+ enhances the magnitude of ethanol inhibition in recombinant channels. We measured the degree of ethanol (50mM) inhibition of N/MG2/2A, N/R2B2B, and N/Q12C/NMDA channels expressed in Xenopus oocytes, comparing effects on four heteromeric subunit combinations; NR1A co-expressed with either NR2A, 2B, 2C, or 2D. At saturating concentrations of agonists (100 μM Mg2+, 100 μM glycine) and glycine toxins in the presence of 12.5 and 3.125 mM Mg2+, the presence or absence of Mg2+ had no effect upon the degree of ethanol sensitivity (μM Mg2+ 19.2% inhibition; 12.5 mM Mg2+/3.125 mM Mg2+ 13.4% for NR2A/B). Reduction or oxidation of the NMDA channel's redox modulatory site by DTT (2mM) or DTNB (1mM) was also ineffective in changing ethanol sensitivity (control: 32% inhibition; DTT: 24%; DTNB: 39% for N/MG2/2A). We have previously shown that NR1A and NR1B homomeric channels were differentially sensitive to ethanol. Whether this difference was also present in heteromeric assemblies, we generated dose response curves for ethanol (25-100mM) to compare the sensitivity of N/R1A/NR2B and N/R1A/NR2C assemblies. The presence of the extra 21 amino acids did not result in a consistent difference in ethanol sensitivity. N/R1A/NR2C assembly, with either splice variant, were significantly less sensitive to ethanol than other combinations. These results indicate that splice variants in heteromeric combinations are not differentially ethanol-sensitive, that the presence of NR2C confers less ethanol sensitivity, and that ethanol sensitivity is independent of both the redox modulatory site and Mg2+ binding site. Supported by NIH grant AA05542.


Haploredox, a therapeutically and functionally related anti-epileptic compound that has recently been shown to have weak inhibitory effects at neuronal NMDA receptors (Fletcher, E. J. and J. F. MacDonald, Ex. J. Pharmacol. 325:291-1993). Using electrical recording techniques we have assayed haploredox on cloned NMDA receptors expressed in Xenopus oocytes, comparing effects on four heteromeric subunit combinations; NR1A co-expressed with either NR2A, 2B, 2C, or 2D. At saturating concentrations of agonists (100 μM Mg2+, 100 μM glycine) and glycine toxins in the presence of 12.5 and 3.125 mM Mg2+, the presence or absence of Mg2+ had no effect upon the degree of ethanol sensitivity (μM Mg2+ 19.2% inhibition; 12.5 mM Mg2+/3.125 mM Mg2+ 13.4% for NR2A/B). Reduction or oxidation of the NMDA channel's redox modulatory site by DTT (2mM) or DTNB (1mM) was also ineffective in changing ethanol sensitivity (control: 32% inhibition; DTT: 24%; DTNB: 39% for N/MG2/2A). We have previously shown that NR1A and NR1B homomeric channels were differentially sensitive to ethanol. Whether this difference was also present in heteromeric assemblies, we generated dose response curves for ethanol (25-100mM) to compare the sensitivity of N/R1A/NR2B and N/R1A/NR2C assemblies. The presence of the extra 21 amino acids did not result in a consistent difference in ethanol sensitivity. N/R1A/NR2C assembly, with either splice variant, were significantly less sensitive to ethanol than other combinations. These results indicate that splice variants in heteromeric combinations are not differentially ethanol-sensitive, that the presence of NR2C confers less ethanol sensitivity, and that ethanol sensitivity is independent of both the redox modulatory site and Mg2+ binding site. Supported by NIH grant AA05542.

470.5 THE DIHYDROPYRIDINE NITRENIDERINE REDUCES N-METHYL-D-ASPARTATE (NMDA)-DEPENDENT SINGLE CHANNEL ACTIVITY BY A MECHANISM CONSISTENT WITH OPEN CHANNEL BLOCK.

G.A. Skrent*, R. E. Tyrwhalt, and H. S. White. Anticonvulsant Drug Development Program; Departments of Pharmacology and Toxicology; and Neurology, University of Utah; Salt Lake City, Utah.

The dihydropyridine nitreniderine was shown by this laboratory to reduce NMDA- (5 μM) and glycine- (1 μM) evoked whole-cell and single-channel currents of cultured rodent cortical neurons in an agonist- and a voltage-dependent manner (Skrent et al, submitted to JPET, 1994). Kinetic analysis of the single-channel data (Vm = 75 mV) for the main conductance (48 ps) showed that the nitreniderine-current ratio - explained in terms of concentration-dependence (30 - 1000 nM) reductions in the frequency of openings and bursts, the average duration of openings and bursts, and the single open duration times. These results suggest that nitreniderine interacts with the open state of the NMDA receptor-associated ion channel. To investigate this possibility further, kinetic models were examination. The simulated experimental data were analyzed and compared for effects on the frequency and duration of NMDA- (5 μM) evoked single-channel openings at several concentrations of nitreniderine (30 - 1000 nM). The simulated data best approximated the experimental data when generated using a model that was similar to one which described the interaction of 4-amino-5-phosphonopentanoic acid (AP4) and NMDA receptors (Macdonald et al, J. Physiol., 410: 479-499, 1991). Consequently, the results support the hypothesis that nitreniderine interacts with NMDA receptors by an open channel mechanism similar to that described for MK-801. Supported by a grant from Mileda, Inc.
470.7

EXCITATORY AMINO ACIDS: PHARMACOLOGY IV

470.8

INCREASED POTENCY OF THE COMPETITIVE NMDA RECEPTOR ANTAGONIST D-AP5 DURING EARLY DEVELOPMENT IN HIPPOCAMPAL PYRAMIDAL CELLS. J.A. Greenblatt, R.J. Bishop, and M.T. Johnson. Tufts University Laboratory & Research, New York State Department of Health, Empire State Plaza, P. O. Box 509, Albany, NY 12201-0500.

NMDA receptor activation plays an important role in the mechanisms of developmental synaptic plasticity. This has prompted investigation of the pharmacological and biochemical properties of NMDA receptor mediated responses and has led to interesting findings in receptor activation with rapid and strong voltage-dependency (IC50 against NMDA 200µM at 70mV, memantine 2.3µM, amantadine 71µM). Similar noncompetitive NMDA receptor blockade was observed in whole cell recordings from freshly dissociated hippocampal neurons (IC50 against NMDA 1.2±0.7µM, memantine 16±3µM). However, in freshly dissociated striatal neurons, recorded under identical conditions, memantine (IC50 12±4µM) was more potent (IC50 0.12µM). A similar relative potency was apparent for the ability of amantadine (100µM) and memantine (10µM) to reduce the amplitude of NMDA receptor-mediated EPSPs and receptor currents. The increase in Vmax apparent in hippocampus in strain compared to other brain regions may underlie its beneficial effects in Parkinson’s disease. Whilst neither memantine (3-10µM) nor amantadine (10-100µM) affected current responses to AMPA (50-100µM) recorded in whole cell mode, similar concentrations of memantine, but not amantadine, weakly potentiated peak responses to AMPA 100µM recorded with the perforated patch technique (memantine 3µM, 11±8% of control, n=7) implying the involvement of secondary messengers. Although this effect was weak and of variable amplitude, similar effects were seen in a separate series of experiments with memantine 10µM against AMPA 50µM (116.3±4.6% of control, n=6). Chronic treatment of superior colliculus cultures for 2 weeks with memantine (3-10µM) had no discernible effect on the acute NMDA antagonist properties of memantine but enhanced the potentiating effects of memantine on peak AMPA responses (127.9±15% of control, n=8). This additional effect of memantine may underlie the reported symptomatic cognitive enhancement seen with this NMDA antagonist in clinical trials of dementia.

470.9

PHARMACOLOGICAL CHARACTERIZATION OF GLUTAMATE BINDING SITE IN RECEPTORS ASSEMBLED FROM 1A, 2A AND 2B NMDA RECEPTOR SUBUNITS. S. J. Lens, E. Greenblatt, and D.B. Pettit. Dept. of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Previous studies in brain suggest pharmacological heterogeneity of the NMDA sensitive glutamate binding site. We have characterized the pharmacological properties of the glutamate binding site assembled from combinations of the 1A, 2A, and 2B NMDA receptor subunits expressed in transfected cell lines. Three-4-Glu-CRP 39653 binding assays. Cells transfected with 1A and 2A, 1A and 2B, and 2A and 2B alone produced saturable, specific binding of 3H-glutamate with Kp of 28±6 mM, 19±7 mM, and 123±21 mM, respectively. No binding was detected in cells transfected with 1A or 2B alone. NMDA inhibited 3H-glutamate binding in 1A:2A, 1A:2B, and 2A:2B combinations with similar IC50 values of 23±14 µM, 14±12 µM, and 12±2 µM, respectively. However, the antagonist inhibition profiles of 3H-glutamate binding differed among the receptors assembled from 1A:2A, 1A:2B, and 2A:2B, with IC50 values of 6±2 µM, 25±13 µM, 57±9 µM for APV, 0±100 µM D-AP5 18.9±18.9 µM, and 0±0.1 µM for CGP 36965, respectively. Specific, saturable binding of 3H-CRP 39653 was detected in cells transfected with 1A and 2A but not detected in cells transfected with 1A and 28, 1A alone, or 2B alone in the absence of Mg++. However, in the presence of 1mM Mg++, the 1A:2B subunit combination displayed specific, saturable 3H-CRP 39653 binding. These results suggest that the N2 type subunits can account for heterogeneity in glutamate antagonist site.

470.10

INTERLEUKIN-1B ANTAGONISM OF NMDA-MEDIATED INCREASES IN INTRACELLULAR CALCIUM IN CULTURED CHICK CORTICAL NEURONS. J.M. Futter, D.G. Lindquist and L.G. Miller. Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, Boston, MA 02111.

Interleukin-1B (IL-1B) is a proinflammatory cytokine produced in the brain which is thought to play a role in both acute and chronic neurodegeneration in the CNS. Since the role of excitatory amino acids in the neurodegenerative process is well known, the present study examines the ability of this neuroactive cytokine to modulate NMDA-mediated increases in intracellular calcium of cultured chick cortical neurons using the fluorescent dye Fura-2. IL-1B alone had no effect on intracellular calcium at the highest concentration used (10 ng/ml). At a concentration of 1 ng/ml, IL-1B significantly attenuated the increase in intracellular calcium seen in the presence of NMDA/glycine. This inhibition was antagonized by 50 µM 6-cyano-7-nitroquinoxaline-2,3-dione (CNXQ), a non-NMDA glutamate receptor antagonist. CNXQ also inhibited the decrease in intracellular calcium caused by IL-1B in the presence of both NMDA/glycine and the endogenous polyanionic spermine. Further experiments suggest that this modulatory effect is specific to IL-1B, inasmuch as the polyanionic cytokine interleukin-6 (IL-6) produced no changes in intracellular calcium in the presence or absence of NMDA/glycine. These data indicate that the mechanism by which IL-1B antagonizes NMDA-mediated increases in intracellular calcium does not occur via the glycine or polyanionic site on the NMDA receptor but may involve non-NMDA receptor subtypes of the glutamate receptor.

470.11

REGIONAL VARIATIONS IN THE PHARMACOLOGY OF NMDA RECEPTOR CHANNEL BLOCKERS. R.H.P. Porter & T.L. Greenblatt. Northeastern University, Boston, MA 02115.

Quantitative receptor autoradiography was used to examine the regional binding characteristics of a diverse group of N-methyl-D-aspartate (NMDA) receptor channel blockers that varied in potency 105-fold. Full competition curves were generated in each of six brain regions for 12 different compounds. MK-801 was the most potent compound tested, with an IC50 of 0.7 µM in the forebrain regions, and 24 mM in the cerebellar granule cell layer (p < 0.05). The Hill slopes of five of the twelve compounds examined were significantly different in cerebellar granule cell layer than in forebrain regions. The fact that the rank order of drug potency in cerebellar regions from highest to lowest supports the notion that cerebellar NMDA receptor ion channels differ biochemically from those in forebrain. These were a general trend that drugs known to be well-tolerated in humans (remacemide and its metabolites, amantadine, batidine and memantine) had lower affinities with several sevenfold less neurobehavioral or psychotomimetic effects. Also, in contrast to MK-801, TCP, PCD, and ketamine, all of these compounds had a significantly higher affinity in the cerebellum than in forebrain regions. Our results suggest that we have identified a new pharmacological microenvironment (cerebellum) where NMDA receptors have a lower affinity and that low affinity (rapid kinetics) and, possibly, subunit specificity may be important determinants of the clinical tolerability for NMDA receptor channel blockers. (National Parkinson Foundation Center of Excellence at the University of Rochester and Fixons Pharmaceuticals)

470.12


It has been shown that chronic administration of 1-aminoacyclopropyranecarboxylic acid (ACPC), a specific ligand for the glycine site of NMDA receptor, results in neuroprotection from ischemic injury and reversible desensitization of NMDA receptors by behavioral responses. This was by NMDA receptor activation (Skolnick et al., Psychopharmacology, 1992, 107:489-496). The hypothesis was made that ACPC could possibly desensitize the NMDA receptor. To test this hypothesis we used Xenopus oocytes injected with rat H-glutamate and recorded NMDA-induced currents using two microelectrode voltage-clamp techniques. In 1980, 11-15% of the glycine measured NMDA current was very small or absent (6.8±2.8 nA vs. 62±1.8 nA in the presence of 100M glycine). Upon addition of 1 µM and 10 µM ACPC the NMDA current increased to 57±1.8 nA (5 cells) and 93±2.3 nA (5 cells), respectively. However in all cells tested, the NMDA current remained stable over 5-15 minutes despite the continued presence of both NMDA and ACPC. Some cells, after initial exposure to ACPC for 5-10 minutes, were upon NMDA isotropic, 100M ACPC: no significant difference was found in the size of the NMDA currents compared to previous measurements. Our results show that ACPC does not desensitize the NMDA receptors, suggesting that some other mechanism may be involved in ACPC-induced neuroprotection.
NEW STRUCTURAL ANALOGUES OF A SELECTIVE NMDA RECEPTOR AGONIST WITH SURPRISING PHARMACOLOGICAL PROPERTIES. T. J. Olesen, K. Flyvbjerg, M. Week, and P.-E. Gjedde. The Danish Society for Psychiatry, DK-2100 Copenhagen, Denmark.

The heterocyclic alanine amino acid (RS)-2-amino-3-oxo-5- methyl-4-oxo-3-thiazolylalanine (AMAA) is a potent and selective N-methyl-D-aspartate (NMDA) receptor agonist. AMAA is an antagonist in these in vitro dose-dependent manner with IC_{50} values of approximately 100 uM. 47 alkaloids of the ASA group of artemisinin have been synthesized and tested in vivo.

Compound I is a selective NMDA receptor agonist with an electrophysiological profile in the rat cortical wedge preparation similar to that of AMAA, though slightly weaker. Surprisingly, compound II is a non-selective antagonist capable of inhibiting both NMDA and AMPA-induced depolarisations in a dose dependent manner with IC_{50} values of approximately 100 uM.

Compound III has been used in this article with the assumption that the high similarity between the compounds makes the data obtained from compound I applicable to compound II.

CHARACTERIZATION OF NMDA/GLYCINE RECEPTOR COMPLEX IN NEONATE RAT SYMPATHETIC PREGANGLIONIC NEURONS. S. W. West* and N. J. Dep. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43614

Whole-cell patch-clamp recordings were made from sympathetic preganglionic neurons (SPNs) in transverse adult spinal cord slices of 10-16 day old rats. Pressure injection of N-Methyl-D-Aspartic acid (NMDA) evoked SPNs in an inward current with a mean amplitude of 110 pA, rise time of 57.0+4.5 pA and decay time of 9.8+2.2 (n=5 experiments) in a non-linear I-V relation with a region of negative slope conductance in the range of -90 to -60 mV in the presence of a linear I-V relation linear in Mg^2+ free solution. The reversal potential, i.e. 0 mV, was the same in both solutions. Superfusion of glycine at low concentration (0.1-100 uM) increased and at higher conc. (0.3-1 mM) decreased the NMDA currents; the holding current and decay time of the NMDA currents were not affected by glycine. 8-

aminocarbonyl derivative (Supported by NS 18710).

NMDA CHANNEL BLOCKADE DURING DEVELOPMENT PRODUCES AGING-SPECIFIC ALTERATIONS IN NMDA RECEPTOR DISTRIBUTION PATTERN. J. He, B. Sircar, Laboratory for Developmental Neuroscience, Departments of Psychiatry and Neurology, Albert Einstein College of Medicine, Bronx, New York.

The NMDA receptor has been shown to play an important role in developmental processes. Phencyclidine (PCP) and PCP-like drugs (MK-801, ketamine) act as non-competitive antagonists at the N-methyl-D-aspartate (NMDA) receptor. We have earlier shown that postnatal PCP treatment in rats produced long-term changes in seizure susceptibility. Here we report results of chronic postnatal PCP treatment on the NMDA receptor distribution pattern. Pups were treated with PCP (5 mg/kg/day) for 11 days from postnatal days 5 to 15, intraperitoneally. Control pups received saline (1 ml/100 g). All rat pups were weaned on postnatal day 21. On postnatal days 21, 40, 60, and 180, separate groups of saline- and PCP-treated rats were sacrificed, their brains removed and immediately frozen in crushed dry ice. Brain sections were incubated with [3H]-MK-801 in the absence and presence of excess nonradioabeled MK-801, washed, dried and juxtaposed against tritium-sensitive film. Quantitative densitometric analysis of the autoradiographic films showed age-specific changes in [3H]-MK-801 distribution patterns following postnatal PCP exposure.

DECREASED MAGNESIUM (Mg^2+) BLOCK OF NMDA MEDIATED EXCITATORY POSTSYNAPTIC POTENTIALS (EPSPs) IN AGED FISCHER 344 RAT HIPPOCAMPUS. M.C. Jaque*, and W.H. Griffith, Dept. of Medical Pharmacol. & Toxicol., Texas A&M Univ Hlth Sci Center, College Station, TX 77843.

The purpose of the present study was to examine age-related changes in the Mg^2+ sensitivity of N-methyl-D-aspartate (NMDA) synaptic potentials in vitro in postnatal hippocampus. We utilized hippocampal CA1 field EPSPs in young (1-2 months) and aged (24-25 months) rats. Synaptic NMDA potentials were studied in isolation in the presence of 10 [uM] DNXQ. In aged rats, EPSP area was measured and all values were normalized to initial EPSPs to the same drug application. It was found that the presence of 10 [uM] DNXQ and Mg^2+ the normalized EPSP area was 1.48±0.17 in young (n=6) and 1.86±0.24 in aged (n=6). When 0.1 [mM] Mg^2+ was added to EPSP area in young increased to 0.78±0.18 while in aged, the area remained almost unchanged at 1.88±0.19. In young, 0.3 mM Mg^2+ increased the area further to 0.39±0.12 while in aged, 0.3 mM Mg^2+ decreased the response to 0.88±0.09. There was a significant difference (p<0.01, independent t-test) between the two age groups at concentrations of 0.1 [mM] and 0.3 [mM] Mg^2+ indicating an age-related decrease in the sensitivity to the Mg^2+ block. Similar experiments were done in the non-NMDA synaptic potential (using 50 [uM] APV instead of DNXQ) resulted in no significant difference in Mg^2+ sensitivity between age groups. These latter results suggest that the age-related changes in NMDA synaptic potentials were due to alterations in postsynaptic sensitivity and not due to changes in neurotransmitter release.

SUPPORTED BY NIH Grant AG07589.
471.1

SPECIFIC DETECTION OF NEUROTENSIN METABOLITES IN THE RAT BRAIN BY IN VIVO MICRODIALYSIS/ELECTROSPRAY MASS SPECTROMETRY

F.E.R. Emmett, H. Caprioli, Analytical Chemistry Ctr., Dept. of Biochemistry & Molecular Biology, Univ. of Texas Medical School, Houston TX 77030

We have developed a highly sensitive microelectrospray mass spectrometry (ES(-)MS) technique in order to detect attomole levels of neuropeptides from brain microdialysis (MD) perfusates. MD samples were desalted on a nano-LC (50 uM i.d.) C18 column at a flow rate of 800 nl/min which was then eluted directly into the mass spectrometer for ES MS analysis. In the present experiments we have studied in vivo neurotenin (NT) metabolites in the striatum. NT is a putative neurotransmitter or neuromodulator in brain and several of the NT-fragments retain biological activity. The detection limit of the nano-LC/MS MS system was less than a total of 100 attomol when synthetic NT was injected.

A MD probe was stereotactically implanted into the striatum of an anaesthetised rat and an infusion of 1 uM NT through the microdialysis probe was performed at 0.3 uM/min. NT was metabolised in vivo and NT-fragments were obtained in the MD perfusate and analyzed by nano-LC/MS. MS spectra showed that NT was metabolised to fragments 1-12, 1-11, 1-10, 1-8, 3-8 and 7-11. We will also show results of experiments which are designed to monitor endogenous NT and its metabolic fragments.

471.2


We have examined the molecular heterogeneity of neuropeptide Y-like immunoreactivity (NPY-LI) in cerebrospinal fluid (CSF). Gel chromatography, not high performance liquid chromatography (HPLC), suggested two NPY immunoreactivity materials in CSF. Both gel chromatography and HPLC revealed three SLI components in CSF; somatostatin 14, somatostatin 28 and a higher molecular weight precursor. We have also measured peptide-LI in control subjects and in patients with various neurologic disorders. We observed a significant reduction in CSF SLI in control subjects over 60 years of age, compared with the younger controls. CSF SLI was significantly decreased in multiple sclerosis (MS), or Guillain-Barre syndrome, compared with that of age-matched control subjects. A reduced concentration of NPY-LI was found in CSF of patients with MS. Our results suggest that (1) the possible heterogeneity of NPY molecules occurs in human CSF, and (2) somatostatin neurons might be more susceptible than NPY neurons in various pathological conditions and aging.

471.3

DOPAMINE D2-RECEPTOR ALCALYLISATION OF NEUROTENSIN RELEASE FROM STRIATUM AND NUCLEUS ACCUMBENS AS MEASURED BY MICRODIALYSIS. J.D. Wagstaff*, J.M. Gibbs and G.R. Hanson, Dept. of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112.

Neurotensin (NT) closely associated with extrapyramidal and limbic dopamine (DA) systems. It is speculated that this peptide neurotransmitter plays an important role in the regulation of these DA pathways and, in turn, NT release is controlled in part by alterations in DA activity. This has not been satisfactorily tested in vivo as the low levels of extracellular NT are difficult to detect consistently. Through a modification of the methods described by Maedent al. (Neurosci. 45:1, 1991), we have developed a highly reliable method of measuring changes in extracellular NT in the striatum and nucleus accumbens in awake rats by in vivo microdialysis. Using this technique, release of NT in vivo was evoked by infusion of a high concentration of potassium (100 mM) through the microdialysis probe; this potassium-evoked release is calcium-dependent. Furthermore, we have examined the effect of stimulating the DA D-2 receptor subtype on NT release. Systemic administration of the D-2 agonist, quipazine (5 mg/kg), produced an increased release of approximately 200% in extracellular NT in the striatum and 40-60% in the nucleus accumbens. This study shows that the DA systems are important factors in controlling NT release in extrapyramidal and limbic DA terminal fields, and that a stimulation of D2 receptors activates associated NT pathways in awake animals. (This work was supported by USPHS grants DA 04222 and DA 00869.)

471.4


We have examined the molecular heterogeneity of neuropeptide Y-like immunoreactivity (NPY-LI) in cerebrospinal fluid (CSF). Gel chromatography, not high performance liquid chromatography (HPLC), suggested two NPY immunoreactive materials in CSF. Both gel chromatography and HPLC revealed three SLI components in CSF; somatostatin 14, somatostatin 28 and a higher molecular weight precursor. We have also measured peptide-LI in control subjects and in patients with various neurologic disorders. We observed a significant reduction in CSF SLI in control subjects over 60 years of age, compared with the younger controls. CSF SLI was significantly decreased in multiple sclerosis (MS), or Guillain-Barre syndrome, compared with that of age-matched control subjects. A reduced concentration of NPY-LI was found in CSF of patients with MS. Our results suggest that (1) the possible heterogeneity of NPY molecules occurs in human CSF, and (2) somatostatin neurons might be more susceptible than NPY neurons in various pathological conditions and aging.

471.5

ACIDIC SULFUR-CONTAINING AMINO ACIDS, \(\gamma\)-GLUTAMYL AND \(\beta\)-APARTYL PEPTIDES IN HUMAN CEREBROSPINAL FLUID

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A method for the determination of acidic amine containing substances in human cerebrospinal fluid (CSF) is presented. The automated technique is based on precolumn derivatisation of primary amines with N,N-dimethylformamide-2-mercaptoethanol (DMF-MCE). Separation was performed by reversed phase HPLC and fluorescence detection. Three different techniques with complementary selectivities were employed in order to minimize false identification. Addition of 2-mercaptoethanol to the CSF was necessary to avoid oxidation of cysteine to cysteine sulfinate. The dominant peptides were \(\gamma\)-glutamylglutamine and glutathione followed by \(\beta\)-aspartylaurine, \(\beta\)-aspartylglycine and \(\gamma\)-glutamylcysteine. The sulfur-containing amino acids cysteine sulfinate, homocysteate, homocysteine sulfinate and sulfo-S-mercaptionate could not be detected.
PARATYROID HORMONE-RELATED PEPTIDE (PTHRP) IS A CEREBELLAR GRANULE CELL-ENRICHED PEPTIDE. IT ACTIVATES THE VOLTAGE-SENSITIVE CALCIUM CHANNEL ACTIVATION. E. H. Holt, B. E. Dresser, A. E. Breden, and M. L. Brown*. Departments of Cellular and Molecular Physiology and Internal Medicine, Wake Forest University, New Haven, CT 06510.

PTHRP-related parathyroid hormone (PTHrP) is a protein secreted widely expressed in mammalian tissues. In the adult rat brain it is expressed in the cerebellum and hippocampus, the brain regions enriched in the cerebellum. PTHrP is encoded by a gene distinct from that of parathyroid hormone (PTH). Although PTHrP bears strong homology to PTH in its N-terminal, the rest of the protein is unique. Primary cultures enriched in PTHrP (P) rat cerebellar neurons are ideal for studying the regulation and function of PTHrP because the highly enriched granule cells possess high levels of the peptide and are devoid of the recombinant. Recently, we have found that the SK-N-SH cells possess the gene to express LHRH. To further characterize the LHRH system in SK-N-SH cells, the concentrations of LHRH and the LHRH mRNA levels were measured during neuronal differentiation. Cells were treated with RA (10 µM) for 1 hr, 2, 4, and 6 days. The amount of LHRH mRNA was significantly higher than controls at all stages of differentiation.

Supported by the URC (ISU) and the Wallace Foundation (College of Pharmacy).


Using microdialysis and a sensitive radioimmunoassay we have studied the in vivo release of the neuropeptide galanin from the ventral hippocampus of freely moving rats. The spontaneous outflow of galanin-like immunoreactivity (GAL-LI) (1.8 fmol/ml/20 min) was dependent on the presence of extracellular Ca2+ and was inhibited by tetrodotoxin (TTX). Evoked release induced by infusion of KCl (60 mM) or veratridine (148 µM) was also Ca2+-dependent. TTX-sensitive release of GAL-LI in the hippocampus. In vitro GAL-LI release, studied in slices of rat ventral hippocampus, was also Ca2+-dependent and increases in a concentration-dependent manner by KCl depolarization. This study provides the first demonstration of in vivo GAL release in the ventral hippocampus. This release is related to the activity of the cholinergic GAL-LI containing cells in the septal diagonal band nucleus.

We thank CNR, Rome, Italy, Convenzione Psicofarmacologia.

PYROGLUTAMYL PEPTIDASE II ACTIVITY IS NOT IN THE PROCESSES OF RAT BULLSPINAL TRH-ERGIC NEURONS. P. Joseph-Bravo*, M. E. Fresen, M. Denero, M. A. Vargas and J. L. Chadi. Instituto de Biotecnología, Universidad Nacional Autónoma de México, A.P.5103, Cuernavaca, Mor., México 62271.

Pyroglutamyl peptide II (PPII) [E.C. 3.4.19.8] is a neuronal ectoenzyme enriched in synapses. Various of its properties, including its narrow specificity, suggest it is involved in the inactivation of TRH released into the synaptic cleft. Because synaptosomes contain pre and postsynaptic elements, we do not know whether the enzyme is pre or postsynaptic. No specific antibodies, irreversible inhibitors or mRNA sequence information have so far been obtained. Therefore, in an attempt to define if it is present in the pre or postsynaptic membrane we induced neuronal degeneration of serotonin-TRHergic cells that project from raphe nuclei to the spinal cord. TRH levels were measured by RIA and PPI activity by a specific radiochemical assay. Two to four weeks after intracerebral injection of 200 µg 5,7-dihydroxytryptamine, the levels of TRH were reduced over 70% in the cervical, thoracic or lumbar regions of spinal cord. In contrast, PPII activity was unaffected in each region. However, 6-8 weeks after injection, a 58-86% increase in activity was detected in the lumbar region. Previous studies had demonstrated that destruction of bullospinal TRH neurons lead to an increase in spinal cord TRH receptors. Our data suggest that PPII is not localized in the processes of bullospinal TRHergic neurons, but it may be in postsynaptic elements. (Supported in part by grant IN-204791 from DGAPA-UNAM.)

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471.13
PURIFICATION AND CHARACTERIZATION OF RAT BRAIN TRANSGlutaminate. H. Ohashi, Y. Itoh, P.J. Rubikovich and Y. Takeuchi, Tsukuba Research Institute, J. Hanyu Pharmaceutical Co., Ltd., Tsukuba 300-33, Japan and Noble Center for Biomedical Research/OMRF, 825 N.E. 13th Street Oklahoma City, OK 73104.

Transglutaminase (TGase) in neuronal system has been suggested to be involved in neurotransmitter release, long term potentiation, Alzheimer's disease, and so forth. In order to have molecular basis on the enzyme in the central nervous system, the enzyme was purified to apparent homogeneity from the centrifugal supernatant of male SD rat brain homogenate, using DEAE ion exchange, heparin affinity, and casein agaroaffinity column chromatographies. The purified enzyme has a molecular weight of 78-kDa on SDS-polyacrylamide gel electrophoresis analysis. The TGase activity was Ca²⁺ dependent (EC₅₀ = 0.38mM), and was maximal in a pH range of 8.0 - 9.5. Km values for putrescine and N,N-dimethylated casset were approximately 0.3mM and 0.05mM, respectively. GTP inhibited the enzyme activity 100-fold more potently than ATP did.

471.14
METABOLIC CONVERSION AND IN VITRO BLOOD-BRAIN BARRIER PERMEABILITY OF AN ENKEPHALIN ANALOG PRO-DRUG. D.J. Groves*, V.S. Han, S.J. Web, T.J. Abercrombie, V.J. Hruby and T.P. Davis, Dept. of Pharmacology and Chemistry, Univ. of Arizona, Tucson, AZ 85724.

To improve blood-brain barrier (BBB) penetration of the delta opioid receptor ligand ([D-Pen₂, D-Pen⁻] Enkephalin (DPDPE), a pro-drug Phε-DPDPPE was synthesized to increase lipophilicity. BBB penetration studies used an in vivo bovine brain microvessel endothelial (BBMEC) model. In vitro conversion was studied using mouse serum and brain homogenized at 37°. To study the rate of conversion to DPDPE, specific aminopeptidase inhibitors were added. To study BBB penetration, BBMECs were grown to a confluent monolayer, suspended and seeded onto membrane filters. Permeability coefficients (P.C) were determined to give the rate of penetration in cm/min. The results showed DPDPE was converted in vivo to DPDPE within 5.0 min in serum and 3.9 min in brain. After adding 6 μM amastatin (aminopeptidase M inhibitor) to the brain homogenate and 50 μM lentil (lichen aminopeptidase inhibitor) to the serum, the half-life of Phε-DPDPPE conversion increased to >50 min. This P.C. (cm/min) for Phε-DPDPPE (62.0 ± 4.1 × 10⁻⁴) was 21% higher than that of its parent compound DPDPE (49.2 ± 2.8 × 10⁻⁴). These results show that the pro-drug Phε-DPDPPE has a longer conversion time in serum than in brain. The results also show that two aminopeptidases are responsible for cleaving Phε-DPDPPE to DPDPE and the addition of phosphatase to the amino terminus of DPDPE increases the BBB penetration to the brain using the BBMEC model of the BBB.

(Supported by N.I.D.A. #DA06284 and N.I.H. - HL47499-09)

471.15
ONO-1603 INHIBITS BRAIN PROLYL OLIGOPEPTIDEASE IN VITRO, IN VIVO AND IN SITU. N. Katsube, H. Maegawa, Y. Kagamimori, M. Yamamoto and H. Akiti. CNS Division, Discovery Research Laboratories, Mitsubishi Research Institute, Ono Pharmaceutical Co., Ltd., Osaka 618, Japan.

ONO-1603, [S] - 1 - [N-(4-chlorobenzyl) succinylamido] - prolyl-leucine - 2-carboxylic acid, inhibited prolyl oligopeptidase (POP) purified from bovine brain in vitro with competitive manner (Ki value, 12 nM). Oral administration of ONO-1603 also inhibited POP of rat brain in several regions in vivo for 2.5 - 4 hrs. To examine the inhibitory effects of ONO-1603 on brain POP in situ, we established a novel method for the evaluation of continuous POP activity in the brain of freely moving rats using the microdialysis technique. 7-N-succinyl-Gly-Pro-Phe-DPDPPE, a specific substrate for POP, was continuously perfused into the hippocampus of the rat brain through the dialysis probe, and the 7-amino-4-methylcoumarin (AMC) level in the dialysate (the product by enzymatic reaction with POP) was monitored. In dialysate, the AMC was continuously and stably appeared, and was decreased by the administration of carboxbenzoyl-proline-proline, a prototype inhibitor of POP, indicating that the AMC level in dialysate reflects the brain POP in situ. Following the oral administration of ONO-1603, the AMC level in dialysate from the hippocampus markedly decreased in a time - and dose-related manner, and the AMC was gradually recovered to the basal level. From these observations, it is demonstrated that ONO-1603 is an orally active inhibitor of the brain POP.

471.16

Two FMRFamide (Fm asleep marginale-like peptide) (FLPs) have been isolated from the nematode Ascaris suum. These are Leu-Asp-Glu-Asp-Arg-Arg-NH₂ which can be abbreviated to KNEF/WFMamide or AFL1 and Leu-Glu-Lys-Tyr-Arg-NH₂, KHEYRFam which we also refer to as AF2. We are presently attempting to purify novel FLPs from the nervous system of the parasitic nematode Haemonchus contortus using reverse phase high pressure liquid chromatography (RP-HPLC). We are monitoring the purification steps with a radio- immunooassay, using an anti-KYSALFMamide antibody (Elphick et al., 1991). We have already demonstrated SALFMamide like immunoreactivity in the nervous system of this parasite (Keating et al., 1993). In this study we have purified a FLP from this organism and identified it as AF2. We have also purified a second immunoreactive peptide which we are presently characterizing. We have shown that AF2 potentiates the effect of acetylcholine in Ascaris dorsal muscle strips and that AF2 induces spontaneous activity in Ascaris dorsal muscle.

These findings show that AF2 has now been shown to exist in a second parasitic nematode and it may be that this peptide is ubiquitous throughout this phylum. Elphick, M.E., Reeve, J.R., Burke, R.D., Thomas, M.C. (1991), Peptide, 12, 455-459.


471.17
CHARACTERIZATION AND PHYSIOLOGICAL FUNCTION OF LUGIN IN THE CENTRAL NERVOUS SYSTEM OF APLYSIA CALIFORNICNA. B.S. Atay, A. Angers and J. DésGroseillers. Department of Biochemistry, University of Montreal, Montreal, Canada, H3C 3J7.

We are investigating the role of neuropeptides in renal physiology. Lower Upper Quadrant (LUQ) neurones and neuron L10 in the central nervous system of Aplysia have been shown to extensively innervate the kidney and regulate renal functions. Three neuropeptide precursor genes (LUQ-1, LUQ-7, and L10-M), which are differentially expressed in these cells, have previously been cloned. However, the nature of the physiologically relevant peptides which they express is still unknown. In this study, metabolic labelling of the LUG cells and RP-HPLC separation of the peptide content allowed us to identify the L5-7 precursor and the processed peptides. Cleavage of the signal peptide occurred between amino acids 23 and 24 of the prepropeptide and generated a propeptide of 89 amino acids. Further processing by endopeptidases at the twin basic residues Lys²-Thr² of the precursor generated a peptide of 76 amino acids, as well as an amidated decapeptide, LUGIN. Immunoneutralization of labeled LUG peptides and RT-PCR on mRNA isolated from neurons LU5-6 allowed us to identify a 4 kDa LUG peptide which could arise by differential splicing. The sequence of LUGIN was determined by amino acid sequencing and by its comigration with the synthetic peptide Ala-Pro-Ser-Tyr-Arg-Pro-Glu-Gly-Arg-Phe-amide in three different RP-HPLC systems. The amidation of LUGIN was further demonstrated by its resistance to carboxypeptidase A digestion. When applied to neuron L10, LUGIN induced a long-lasting inhibition of L10 activity. No other neurones in the abdominal ganglion, when tested at random, responded to LUGIN. Finally, LUGIN has also been purified from the kidney.
PEPTIDES: BIOSYNTHESIS AND PROCESSING

472.1
CHARACTERIZATION OF cDNAs ENCODING APYASIS CALIFORNICA FURIN AND PC2: G.T. NAGLE, A.T. GARCIA, S.L. KNIGHT, E. FORGHAM AND A. ROBINS. Mark H. T. S. Department of Anatomy and Neurosciences, Humanities & Basic Sciences, and Human Biological Chemistry & Genetics, University of Texas Medical Branch, Galveston, TX 77555.

The neuropeptide bag cells and exocrine atrial gland of Apysa californica express genes belonging to the prohormone (proH) family and produce the mating precursor at mono-, di-, tri-, and tetrasaccharides. Some of these changes may result from proteolytic activity of a furin-like or PC2-like enzyme; these enzymes have been identified in organisms diverse as yeasts and humans. The atrial gland is particularly interesting because the organ produces large (milligram) amounts of ELH-related peptides and may be a relatively rich source of prohormone processing enzymes. The proH DNA library was screened and a probe encoding a furin-related amino acid was isolated. The open reading frame is a furin-related PC2 product from the bag cells. The library was also screened using an L-protein 2 (pro PC2) (provided by A.B. Smit and W.P.M. Gernert, Holland) and a clone encoding a PC2-like protein was isolated. Both clones contain the long intron regions (UTR's). The 3'-UTR's contain multiple polyadenylation sites which could be used to generate multiple mRNAs. Furthermore, the 3'-UTR's also contain relatively long microsatellite repeat sequences (CA) and (TG). The eventual goal of these studies is to elucidate the potential role of these enzymes in prohormone processing in Apysa. Supported by NIH Grant NS 29261.

472.2
Yeast Aspartic Protease 3 Cleaves Prohormones at Selective Paired and Mono-Basic Residues with Preference for a Lys/Arg ResidueUpstream from the Cleavage site, N. S. Casellas1, 2, H.-C. Chen1, M. C. Brindell2 and Y. Peng Lab1, 2. From the Lab of Developmental Neurobiology, and Endocrin. and Reprod. Research Branch, NICHD, NIH, Bethesda, MD 20892 and the Uniformed Services University of the Health Sciences, Bethesda, MD 20814 and the Dept. Pharmacological and Physiological Sciences, St. Louis University Medical Center, St. Louis, MO 63104.

The novel yeast aspartic protease 3 (YAP3) was overexpressed by induction of yeast strain BFB501 that was transformed with a plasmid containing the YAP3 gene under the control of the galactose inducible promoter. YAP3 was secreted in the growth medium and was partially purified by concanavalin A affinity chromatography. The specificity of YAP3 was studied using a number of polypeptide substrates: porcine cholesytokinin 33 (CCK33), porcine dynorphin A(1-11), porcine dynorphin B(n1-13) and bovine proenkephalin. Analysis of the products generated from each substrate demonstrated that YAP3 cleaved at selective paired and mono-basic residues generating CCK8 and CCK22 from CCK33, Leu-enkephalin and Leu-enkephalin-Arg from dynorphin A and dynorphin B respectively and an extended form of the same from proinsulin. Cleavage at mono-basic residues occurred only when a Lys/Arg was present in the -5 or -6 position relative to the cleavage site.

472.3
PROHORMONE CONVERTASE 2 IS EXPRESSED IN PROENKEPHALIN CONTAINING NEURONS IN DEVELOPING AND ADULT RAT CEREBELLUM. Y. P. Lu, A.M. Bamberger, W.P. Hayes, and L.D. Fish. Lab. of Developmental Neurobiology, NICHD NIH, Bethesda, MD 20892.

The enzymatic processing of peptide precursors at basic amino acids is a key step in neuropeptide biosynthesis. Prohormone convertase 2 (PC2) may participate in such processing. We have examined the distribution of PC2 mRNA in rat cerebellum during postnatal development and in adult and compared it with that of proenkephalin mRNA and enkephalin precursor. Using in situ hybridization, we showed that PC2 mRNA was expressed in a distinct layer pattern in adult rat cerebellum. The Purkinje cell layer expressed very high levels of PC2, while the granular cell layer showed low levels of expression. High expression of PC2 mRNA was also found in Golgi cells. Similarily, proenkephalin mRNA was detected in Purkinje cells and Golgi cells during cerebellar development, PC2 and proenkephalin mRNAs were present as early as postnatal day 1 (P1). With age, there was a gradual increase in the expression of PC2 mRNA in Purkinje and Golgi cells through adult, which correlated well with that of proenkephalin mRNA. Interestingly, a transient expression of PC2 mRNA was observed in granule cells, which appeared as early as P1 and increased to a maximum at P28, an age corresponding to cell division and maturation. Further, in situ hybridization combined with immunocytochemistry, a cellular coexistence of PC2 mRNA and proenkephalin was found in enkephalin expressing Purkinje cells and Golgi cells. These findings implicate the role of PC2 in neuronal proenkephalin processing in both developing and adult cerebellum.

472.5
IDENTIFICATION OF AT, LIGANDS IN THE RAT BRAIN AND DEMONSTRATION OF IN vivo SYNTHESIS INDEPENDENT OF ANGIOTENSIN CONVERTING ENZYME ACTIVITY. B.A. Mangal, A.C. Boll, J.W. Harding and J.W. Wright. Program in Neuroscience and Department of Veterinary and Comparative Anatomy, Pharmacology, Physiology and Psychology, Washington State University, Pullman, WA 99163.

We have recently discovered a unique angiotensin AT receptor, site, assumed to bind endogenous angiotensin IV. We have provided definitive evidence regarding the identity of the naturally occurring ligand acting at this site. Utilizing asetic acid peptide extraction, High Performance Liquid Chromatography separation and Radioimmunounassay detection, we have identified and quantified angiotensin IV-like peptides in rat cerebellum, cortex, striatum, hippocampus, thalamus and hypothalamus with high binding affinity for this AT receptor. In addition, in vivo metabolism studies of radioactively labelled peptides in rat cerebellum, cortex, striatum, hippocampus, thalamus and hypothalamus have been performed to determine the rate of metabolism of the endogenous angiotensin IV-like peptides. In vivo metabolism studies of cerebellum homogenates have confirmed these findings. These studies indicate the presence of high affinity endogenous AT ligands, and in formation of new AT independent angiotensin pathways. The possibility of AT, activation playing a role in the observed effects of captopril therapy, especially on mood and cognition may provide an insight into the mechanism of ACE inhibitors.

472.6
ANATOMICAL RELATIONSHIP BETWEEN GENES ENCODING PROHORMONE CONVERTASIES AND PRO-TRH IN ADULT RAT BRAIN: IMPLICATION IN DIFFERENTIAL PROCESSING OF PRO-TRH. L. P. Par, W. Ma, L.L. Barker and Y. P. A. Lab. of Neuro, NICHD and LNP, NINDS, NIH, Bethesda, MD 20892.

Pro-TRH in the CNS is differentially processed at paired-basic residues to generate several copies of TRH along with other pro-neuropeptides. The mechanism for such differential processing is not yet known. Recently, prohormone convertases (PC1 and PC2), responsible for paired-basic residue cleavages, are found to be expressed in a highly specific and tissue-specific manner. We have used in situ hybridization histochemistry to analyse the anatomical relationship between genes encoding PC1, PC2, and pro-TRH in rat brain. PC1 and PC2 mRNA expressing cells were widely distributed throughout the brain. Expression levels of PC1 and PC2 mRNAs differed in various brain regions with PC2 showing a broader distribution. Regions with high concentrations of PC1 and PC2 cells demonstrated a good correspondence with that of pro-TRH cells. They included olfactory bulb, forebrain areas, hippocampus, hypothalamic nuclei, and several nuclei in brainstem. However, not all brain areas with high levels of prepro-TRH mRNA had high levels of PC1 and PC2 mRNAs. For example, in thalamic reticular nucleus prepro-TRH mRNA was expressed, but neither PC2 mRNA nor PC1 mRNA was detectable. By performing double-labeling in situ hybridization histochemistry, a cellular coexistence of PC1 or PC2 mRNAs with prepro-TRH mRNA was found in a different manner in several brain regions. These results suggest that the different expression patterns of PC1 and PC2 in pro-TRH neurons may be responsible for the differential processing of pro-TRH in various rat brain regions.
472.7 TISSUE DISTRIBUTION AND DEVELOPMENTAL STUDY OF APLYSIA PRO-HORMONE CONVERTASE PC2. T. Oimine* and V.F. Castellucci. Lab. of Neurobiology and Behavior, ICRM, Univ. de Montréal, Montréal, Québec, H2W 1R7.

The nervous system of the marine mollusc Aplysia californica contains many pro-opiocortin peptides which must be processed by pro-opiocortin convertase at multibasic sites to release their bioactive moieties. We have recently characterized the cDNA structure of a subtilisin-like serine protease of the nervous system of Aplysia (aPC2) which appears to be a homolog of the vertebrate PC2, of the pro-hormone convertase family. In-situ hybridization to a large number of mRNAs in the nervous system. Study of the tissue distribution of the aPC2 transcript by Northern blot analysis also revealed its presence in the atrial gland as well as in muscle. This last result was also confirmed by in-situ hybridization. Northern blot analysis of early stages of Aplysia development reveals the presence of a shorter transcript. Thus, aPC2 seems to have a larger distribution in Aplysia than in vertebrates where it seems to be restricted to neuroendocrine tissues. Furthermore, different aPC2 transcripts seem present during development. Funded by MRC of Canada (MT-12099).

472.8 REGULATION OF CHOLECYSTOKININ mRNA BY RETINIC Acid AND D-ADRENERGIC AGENTS IN CELL LINES. B. L. Mansie-Fornal*, J. W. Burns, and T.P. Barlow. Department of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ.

Regulation of cholecystokinin (CCK) mRNA expression was studied in two CCK expressing cell lines, the human neuroendocrine cell line SK-N-MCIX and a rat medullary thyroid cell line WE 42. SK-N-MCIX and WE 42 express CCK mRNA at high levels and perform post-translational processing of CCK (Kontings et al., Neuropeptides 25, 1993; Verbeck and Barbour, FERS 268, 1990). WE 42 express CCK mRNA at high levels of CCK and process pre-CCK to biologically active forms (Beifeld, Peptides 13, 1992). To examine the effect of D-adrenergic agents on CCK mRNA we used the D-adrenergic agonist isoproterenol. We also treated cells with a combination of isoproterenol and the D-adrenergic antagonist propranolol. The effect of retinoic acid on the modulation of CCK gene expression was also examined. Messenger RNA levels were quantitated using Northern blot analysis with a cRNA CCK probe. Isoproterenol significantly increased CCK mRNA levels in SK-N-MCIX cells after 6, 12 and 24 hr treatments, the increase was blocked when isoproterenol was combined with propranolol. In WE 42 cells isoproterenol increased CCK mRNA levels after 6 hrs, although not significantly, the increase was no longer present after 12 hrs. Retinoic acid had no effect on CCK mRNA levels in WE 42 cells after 6 or 12 hr treatments however, there were significant decreases in CCK mRNA levels in SK-N-MCIX cells after 6, 12 and 24 hrs. Our results indicate that CCK mRNA levels may be differentially regulated by D-adrenergic agents and retinoic acid. (Supported by N.I.H. Grant DK 36289 and M142600 and MRC Grants MT11268 and PG2.)


Nigrostriatal dopaminergic neurons are known to regulate enkephalin and substance P gene expression in the striatum. We studied possible modulation of striatal peptide mRNA levels by corticostriatal glutamate input following disruption of glutamate neurons, glutamate release and receptor binding. Glutamatergic afferents were bilaterally lesioned by partial frontoparietal ablation of the cerebral cortex, glutamate release was inhibited by laminotomie treatment (3 and 20 mg/kg, i.p., once a day) and NMDA receptors were blocked by microintrastryatal injection of CPP (1 and 1.2 mg/kg). Striatal enkephalin and substance P mRNA levels were measured one week following the start of the experiment by using in situ hybridization histochemistry according to Young et al. (1996). Coronal brain sections (12 μm) cut at three different rostrocaudal levels of the striatum were incubated with 35S-labelled oligonucleotide probes, exposed to X-ray film and average optical densities were analyzed by computarized densitometry.

Following decontraction both enkephalin and substance P mRNA levels were decreased particularly in dorsolateral part of the rostral striatum (17.5-25.8%). The overall hybridization signal for striatal peptide mRNA was not affected by subchronic administration of lamotrigine, NMDA receptor blocker by CPP infusion decreased enkephalin (14.4-34.6%) and substance P mRNA levels (13.9-38.2%) in a dose-dependent manner in the striatum. It seems that enkephalin and substance P gene expression in the rat striatum is regulated by glutamate in addition to the well-established dopaminergic regulation.

472.10 ISOLATION OF THE GC-RICH, TATA-LESS PROMOTOR FOR THE GENE ENCODING THE BIFUNCATIONAL ENZYME PAM. Hand. T.A. Martin, R.E. Epler, B.A. * Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

PAM (peptidylglycine ω-amidating monoxygenase) is a bifunctional enzyme catalyzing the 2-step conversion of glycine-CPG, to the ODQ-terminal neuronendocrine peptides to ω-amidated bioactive products. The 27 protein coding exons of the single copy PAM gene were isolated from rat genomic libraries and span more than 160 kb of genomic DNA. Southern analysis of PAM is tightly regulated in a tissue and developmentally specific manner, isolation of the promoter was undertaken. Exon 2 of the PAM gene contains 3015 of 5'-UTR and the 5' most intron, exon and primer extension reactions indicated that the longest cDNA clone isolated lacked the most 5'-end of PAM. Using RACE, products isolated from intron and at amino acid of PAM (NRL) contained 98 of upstream coding sequence preceding Exon 2. Screening a genomic library yielded a clone containing 69 of the sequence in the RACE product (Exon 1) flanked by conserved intron junction sequences. Subsequent screening of a cDNA library (made from the NLSs of haploidoser-treated rats) with a 5'-specific probe revealed a clone having an additional 21 of upstream coding sequence. Exon 0 was identified by screening the genomic library with an oligomer complimentary to the most 5'-sequence. The clone identified contained the 50 of Exons 0 followed at the 3' end by a consensus exon/intron junction sequence. A highly GC-rich sequence having several consensus SP1 binding sites but lacking TATA or CAAT sequences preceded Exon 0. Two kb of the upstream sequence (PAM-US) was subcloned into a reporter vector (pGL2, Promega) in the sense or antisense orientation and transiently transfected into GH3 cells, which endogenously express PAM. The PAM-US sense construct yielded levels of luciferase expression at least 150 times that of the antisense construct. Support DK 32949.


Posttranslational processing of peptide precursors frequently includes amidation of C-terminal glycine residues. Several lines of evidence from the proteolytic cleavage of proenkephalin (PENK) by peptidylglycine ω-amidating monoxygenase (PAM) to evaluate the contribution of this postpeptidase modification of PENK cleavage. The PENK is first detected at a level in the null of transnet arteries at e10. By e12 its expression has expanded to cover the aortic sac and common ventricular chamber, which overlaps with furin expression. In the nervous system, PE expression is confined to the developing neural plate and is particularly abundant in the thalamic, striatal, pont, medulla, and the spinal cord from e14 onward. At these stages, PC2 is expressed widely in the CNS and overlaps extensively with PE expression, whereas PC1 expression is primarily associated with PE expression only in the medulla and in late gestational stages PE expression is also detected at a high level in a variety of mesenchymal tissues, which overlaps with furin expression. Together these data suggest that PE expression in development may be processed in a tissue-specific manner by different sets of proteases. In the nervous system PE may be processed extracellularly by PC2 and to a small extent by PC1, generating enkephalin pentapeptides or smaller ECPs. PE in the peripheral tissues, in contrast, may be processed extracellularly by a GTPase cleaving endoprotease to form a detectable free enkephalin in tissues outside of nervous system. Supported by DA-08622 (IEP).


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AN IN VITRO MODEL FOR THE STUDY OF NEUROTRANSMITTER REGULATION OF HYPOthalamic GENE EXPRESSION: RNA FROM VITRO AND VIVO CELLS.

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Karolinska Institute Clinical Neuroscience, Geriatric Medicine, Novum KCF, Huddinge, Sweden.

Initial screening of different human neuroblastoma cell lines for expression of Somatostatin immunoactivity (SS-ir) and prepro-SS mRNA revealed only one candidate, the neuroblastoma LA-N-2 cell line. Extracted SS-ir material from LA-N-2 cells was compared with SS-ir extracted material from rat hypothalamus after gel filtration. The SS-ir in each fraction was analyzed with RIA revealing three major peaks of SS-forms in the rat hypothalamic extract. The prepro-SS mRNA peak with SS-14, the second peak coeluted with SS-28 and the third peak was found close to thevoid volume and corresponded to an estimated molecular weight of 15 kDa. In contrast the SS-ir extracted from the LA-N-2 cell line showed a quite different elution pattern. Three peaks of SS-ir were measurable, one which corresponded in elution position to synthetic SS-28 and two others which corresponded to approximately 8 kDa and 15 kDa. No immunoreactivity was observed in the fractions coeluting with synthetic SS-14. Total cellular RNA from LA-N-2 cells was prepared and examined for the expression of prepro-SS mRNA by Northern blot analysis, revealing the presence of a single prepro-SS mRNA of about 850 nucleotides in LA-N-2 cells. In addition this cell line is devoid of cholinergic and cholinergic systems interact. Such interactions are of interest considering the marked reduction of these two transmitters in cognitive disorders including Alzheimer's disease.

471.15 NEURAL SPECIFIC PROCESSING OF VGF PROTEIN LED TO THE PRODUCTION AND REGULATED SECRETION OF PEPTIDES DERIVED FROM ITS C-TERMINUS. A.M. Binkall* N. Cahn, A. Levy, G.L. Ferris, M.T. Cotti, E. Tran.

Institute of Neurobiology, UMR, C. Marx 15, Rome, Italy; §Dept. Citomorphology, University of Cagliari; †Dept. Experimental Medicine, University of Tor Vergata, Rome.

VGF, a protein of 617 amino acids, has a restricted expression in sub-populations of neuronal and endocrine cells. By the use of polyclonal antibodies directed against different regions of this domain of VGF we demonstrated that in vivo as well as in vitro, neural cells are able to cleave this protein in smaller peptides. This processing is not observed in transfected PC12 cells, a line established from a rat pheochromocytoma, are unable to process VGF, but acquire the ability to mature this protein upon neuronal differentiation in response to NGF. In primary cultures of cerebellum granule cells in vitro maturation correlates with increased processing of VGF. The major products of the VGF cleavage are a polypeptide of apparent molecular weight 20 kDa and a doublet in the 14-10 kDa range which are derived from the COOH-terminal of the protein. These products are enriched in preparation of dense core vesicles from neuronal cells and are preferentially secreted upon depolarization in neuronal primary culture and cell lines. We suggest that these VGF-derived peptides are the biologically relevant species which may play a role in neural communication.

Supported by a grant from F.P. Invecchiamento C.N.R.

Differential effects on neostriatal neuropeptide mRNA abundance by excitatory amino acid receptors: H. Villagas, L. Lacroix*, B. Mecquet and Y. Angus*, Lab of Neuroendocrinology, Rockefeller University and Dept. of Biological Sciences, Hunter College CUNY, New York, N.Y. 10021.

Cortical, thalamic and amygdaloid inputs to the neostriatum employ excitatory amino acids as transmitters. These inputs play a central role in excitation of neostriatal neurons, most of which utilize neuropeptides as transmitters and/or modulators. The effects of NMDA and AMPA/kainate receptor blockade on neuropeptides (mainly preproenkephalin (PPE), proctoxykinin (PK) and proopinephrin (P) subhks in the caudate-putamen (CPU) and nucleus accumbens (NAC) were assessed with [H-3]enkephalin and [3H]enkephalin (NMDA and AMPA/kainate antagonists, respectively). Daily systemic injections with [3H]enkephalin for seven consecutive days increased the abundance of all three neuropeptides mRNA in the caudate-putamen and nucleus accumbens. The abundance was increased in the anterior CPU (26%), dorsal and ventral CPU (46% and 39%, respectively) but was unaffected in the NAC. (2) [3H]enkephalin was increased in the NAC (33%) as well as caudate (27%), dorsal (43%) and ventral CPU (67%). (3) Pre mRNA was elevated in dorsal and ventral regions of the CPU (24% and 24%), but not in the anterior CPU (50%). In the NAC Pre mRNA was increased only at the higher doses (0.1 mg/kg) of [3H]enkephalin. In contrast, systemic injections of [3H]-enkephalin for seven consecutive days decreased PE and Pre mRNA abundance approximately 30% below control day in both the CPU and the NAC. These observations demonstrate that NMDA and AMPA/kainate receptors differentially modulate neuropeptide expression in the neostriatun of the rat brain.

471.16 ALTERED PRODUCTION OF PROHORMONE CONVERTASE 2 (PC2) mRNA IN THE ARC (ARC) OF MIDDLE AGED C57BL/6J MICE. D. Kushi,*, H. Miller, M. W. Silbaid and R. Day.

Departments of Experimental Medicine, Obstetrics and Gynecology, Anatomy, and Center for Studies on Aging, McGill University; Clinical Research Institute of Montreal, Montreal, Quebec, Canada.

We have previously reported a significant increase in the expression of the β-endorphin (β-endorphin(1-31)) and (1-26) forms in ARC of middle aged irregularly cycling female C57BL/6J mice (3-months) which was correlated with the appearance of subunits/kinin family, mediated cleavage of β-endorphin(1-31) to β-endorphin(1-27). The objective of this study was to determine if an increase in PC2 expression is associated with the observed changes in β-endorphin processing. Primer extension analysis of a cDNA antisense CRNA probe to mouse PC2. ARC of young (4-5mo) normally cycling (n=5) and middle aged (12-13mo) irregularly cycling (n=5) female mice in distreus of the estrus cycle were pooled for Northern blot analysis. A significant (p<0.01) increase in the PC2 mRNA observed in ARC of middle aged animals as compared to young mice. For in situ hybridization, two sections each from five ARC subregions were examined in both young (n=3) and middle aged (n=3) mice. Film autoradiography of in situ hybridization revealed a significant proportional increases in mid ARC PC2 mRNA (p<0.05 ANOVA) of middle aged mice vs. young females. These data suggest that there is an increase in the level of PC2 mRNA in ARC in middle aged female mice, which may account, at least in part, for altered processing of β-endorphin in middle aged irregularly cycling female mice.

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471.17 TRANSCRIPTIONAL REGULATION OF THE NGF GENE IN RESPONSE TO NGF.

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Nerve Growth Factor has been reported to regulate the expression of the NGF gene in PC12 cells. The study of the intracellular mechanisms involved in mediating such effects on NGF is important as it may provide a basis for understanding how neuronal cells integrate a variety of stimuli in order to produce a coordinated response. Using a CAT reporter system, we have shown that an AP1 consensus sequence is responsible for the NGF-induced transcriptional activity of the NGF gene. However, a residual response to NGF was observed after the deletion of this AP1 site, suggesting that other sequences are also involved in the residual response. This residual response is mediated through both PKC and PKA dependent mechanisms. Specific inhibitors such as calphostin C (PKC) and H-89 (PKA) were shown to block only partially this residual response to NGF when applied alone, whereas combined application of both inhibitors completely blocked it. Two consensus sites for AP-2 transcription factors which are also present in the NGF promoter region, appear to be responsible for the remaining response to NGF. The deletion of these three sequences completely abolished the response to NGF. Basal levels of expression of the NGF gene in PC12 cells were also found to be mainly dependent on the two adjacent AP-2 sites.

Funded by the Wellcome Trust.

Mutations in DNA underlie carcinogenesis, inherited pathology and aging and are generally thought to be introduced during meiosis and mitosis. Here we report that in post-mitotic neurons specific frameshift mutations occur at high frequency. These mutations were identified in vasopressin transcripts in magnocellular neurons of the vasopressin-deficient hormone Brattleboro rat and predominatingly consist of a GA deletion at GAGAG-motifs. In homonymous Brattleboro rats substituted with vasopressin for 40 weeks and displaying a normalized water balance a 25% reduction in the number of vasopressin cells displaying a GA deletion was found. This indicates that the diseased state of the Brattleboro rat, resulting in a permanent activation of vasopressin neurons, enhanced the mutational rate. Using antibodies against peptides predicted from the +1 reading frame of vasopressin mRNA, immunochemical evidence was obtained for similar events in the hypothalamus of wild-type rats and human. These data have revealed hitherto unrecognized somatic mutations in non-dividing neurons. Such mutations are not restricted to the Brattleboro rat and may occur more widely in neuronal systems affecting other neuronal genes. Evidence for frameshift mutations in other genes will be presented.

CATECHOLAMINES: MEASUREMENTS, SPECT, LESIONS—IMMEDIATE EARLY GENES


Somatodendritic release of dopamine (DA) from the substantia nigra (SN) and ventral tegmental area (VTA) may represent a non-classical form of signaling in the nigrostriatal dopamine and fast-scan cyclic voltammetry voltammetry, with 8 μm carbon fiber microelectrodes to detect endogenous dopamine efflux in situ from tyrosine-hydroxylase (TH) positive neurons. Three pulses were monitored during local electrical stimulation, using a train of 100 pulses delivered at 10 Hz. Signals attributable to DA were identified on the basis of anatomical, electrochemical and pharmaceutical criteria. The response exhibited site-specific variation that correlated well with TH staining: release was significantly higher in VTA (1.04 ± 0.19 μM) than in SN pars compacta (0.52 ± 0.05 μM), which was in turn significantly higher than in SN pars reticulata (0.34 ± 0.03 μM).

The voltammetric of the released substance had oxidation and reduction peak potentials that corresponded to those of DA and the signal evoked in the DA-rich striatum, and were distinguishable from those of 5-HT. The response had apparently no contribution from DOPAC, since it was unaffected by 20 μM pargyline. Electrically-stimulated DA release was Ca2+-dependent, but TTX-independent. The selective DA uptake blocker, GBR 12909 significantly increased the response (260% of control), while desipramine, a selective inhibitor of NE uptake had no significant effect. We conclude that the direct monitoring of stimulat-DA release in situ with carbon fiber electrodes can provide new insights into the mechanism and function of dendritic release. Supported by Bristol-Meyers Squibb and NIH grant NS-28480.

MULTICOMPONENT ANALYSES OF MONOAMINES AND RELATED COMPOUNDS IN CEREBROSPINAL FLUID BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) COUPLED WITH ELECTROCHEMICAL ARRAY DETECTORS. C.J. Hope*, P. Huetli, E. Isler, D. Kirch* and G.A. Gerhardt. Dept. of Psychiatry, Univ. of Colorado Health Sciences Center, Denver, CO, NIMH, Washington D.C., and Medical College of Georgia, Augusta, GA.

Recent studies in our laboratory have involved the detailed analyses of monoamine neurotransmitters and related species in CSF using HPLC coupled with multichannel coulometric electrochemical array detectors. Such approaches allow for the analysis of up to 30 neurochemicals in a single sample. Frozen CSF samples (0.5 ml) are thawed, diluted to 1 ml with mobile phase, filtered and divided into two 0.5 ml fractions. An isocratic HPLC system consisting of a single pump, C18 reverse phase column (4.6 mm x 100 mm, 3 μm particles, Hypersil; Keystone) and a multi-channel electrochemical array detector (ESA 1100A, ELS oxidation and E2 reduction using a 5011 cell) is used to measure levels of dopamine, norepinephrine, DOPAC and other species using a pH 4.0 cirtate-acetate mobile phase with 4.7% MeOH and 0.33-0.37 mM OS. A gradient elution with 16 millieuqals with 20 plasma electrochemical detectors set at increasing potentials ranging from 0 mV to 1200 mV (CESA, ESA Inc.) is used for the measurement of tyrosine, tryptophan, homovanillic acid and up to 20 other neurotransmitter and related compounds. Separations performed with the CESA are carried out with a combination of both phenyl (4.6 mm x 150 mm, 5 μm particles, BDS Hypersil Phenyl, Keystone) and cyanopropyl (4.6 mm x 50 mm, 5 μm particles, Keystone) columns using a gradient elution with a pH 3.0 phosphate buffer containing 0.17-5% ethanol. The use of the electrochemical array chromatography systems for studies of monkey and human CSF samples will be presented.

REDUCTION/OXIDATION RATIOS AND NEUROTRANSMITTER IDENTITY: EFFECT OF NAFION COATING THICKNESS. B.T. Menlooo*, C. R. Haims, Colliano & M. C. Davidson. Dept. of Psychiatry, UC San Diego School of Medicine, La Jolla, CA 92039 and Institute for Neuroscience, Univ. of Oregon, Eugene, OR 97403.

Studies utilizing in vivo voltammetry to investigate the evolved release of catecholamines comprising neurotransmitter and nerve terminals. The electrical signals from neurons may be detected chronovoltammetrically by applying a positive potential step (vs. an Ag/AgCl reference electrode) to the working electrode to oxidize electroactive molecules. The reverse current flow generated by the return of the potential to its previous value reduces the oxidized electroactive species. The ratio of the reduction to oxidation current at the maximum oxidation current is termed the reduction oxidation (RO) ratio. It has been suggested that the RO value gives some indication of the identity of the major neurotransmitter (Gruion et al., Neurosci. 29: 57-64, 1989).

We have recently found that the RO ratio may be related to the thickness of the electrode's Nafion coating. Nafion was applied by simple immersion or with electric current. Our results showed that as the amount of Nafion coating increased, the RO ratio also increased when the only substance added to the calibration medium was norepinephrine, dopamine, or serotonin. Moreover, increases in the duration of applied voltage also produced systematic changes in RO ratio. A three fold-increase in plating time caused a comparable increase in RO ratio for norepinephrine. While dopamine may usually exhibit a larger RO ratio than norepinephrine or serotonin, that ratio may be different for each electrode. Also, since the electrode is in the brain, the nafion coating may begin to thin and RO ratios will change. Thus, RO ratios depend on the neurotransmitter present and the properties of the detecting electrode. Supported by the J. S. McDonnell Foundation and Pew Memorial Trust and NIMH Grant 5T32MH19547 from the NIMH Minority Neuroscience Fellowship Program.

A STABLE LOW MAINTENANCE HPLC FOR THE SIMULTANEOUS ANALYSIS OF CATECHOLAMINES, INDOLES AND THEIR METABOLITES. J.D. Harvey-White, and J.J. Kopin*. Clinical Neurosciences Branch, National Institute of Neurological Disorders and Stroke, 9000 Rockville Pike, Bethesda, MD 20892.

Procedures for obtaining a consistently stable, sensitive, and low maintenance HPLC with electrochemical detection for the simultaneous analysis of norepinephrine, epinephrine, dopamine, dihydroxyphenylalanine(L-DOPA), dihydroxyphenylacetic acid (DOPAC), 4-hydroxy-3-methoxyphenylalanine (OMDOPA), 4-hydroxy-3-methoxyphenylethylamine, homovanillic acid(HVA), and 6-hydroxydopamineacetic acid(6HIAA), and serotonin(SHT) are described. Conditions to meet different experimental needs for optimal selectivity, and sensitivity have been determined: 1 - Heptanesulfonic acid(HSA) is used as the ion-pairing agent. The retention (RT) time for HVA and SHT are inversely related to the concentration of HSA (all other compounds are proportionately related). The RT of L-DOPA and 3-MOMDA (relative to other compounds) are greatly increased as pH is decreased. Resistance to complete drying of the mobile phase(MP) facilitates pump maintenance, the MP can be recycled for months, and the columns last 1 to 2 years. Addition of 1% EDTA/0.02% ethanol to dialysates stabilized all compounds of interest: with 0.1M perchloric acid tissue homogenates this addition stabilized DOPAC and SHT, and increased the stability of 6HIAA.
CATECHOLAMINES: MEASUREMENTS, SPECT, LESIONS—IMMEDIATE EARLY GENES

473.5

A comparative SPECT evaluation of the regional uptake of 28-carboxylpropyl-201-Tc (ICPIT) and 28-carboxylpropyl-324-thyroxine (8-CIT) was performed to assess the improved specificity of ICPIT over 8-CIT for the dopamine (DA) transporters. For ICPIT and 8-CIT five single bolus injections (80 mg/kg) was given every 10 min (total time ranging from 70 to 80 min) into 5-15 mcI injected rats, were completed in 3 baboons (Papio anubis, 10 kg). Peripheral metabolism of the test drugs was similar as demonstrated by the respective clearances, 175±23 ml/min (mean ± SD) for ICPIT and 134±9 ml/min for 8-CIT. The SPECT images utilized R0s over striatum (which reflect DA transporters), midbrain (previously shown for 8-CIT to reflect primarily arocholinergic transporters), and the cerebellar cortex (as a non specific uptake). The time to peak specific striatal uptake (striatal minus cerebellar activity) was similar for ICPIT and 8-CIT (37±5±1 min, respectively). At time of peak specific activity, striatal to cerebellar ratios were 2.8±1.3 for ICPIT and 7.2±0.8 for 8-CIT. However, the normalized striatal uptake values (mean±SE) were similar to those of both ICPIT (5.3±0.8) and ICPIT (3.1±0.2, respectively). In contrast, the normalized nonspecific activity measured in cerebellum was higher for ICPIT (1.8±0.4) than for 8-CIT (0.4±0.3). In conclusion, ICPIT demonstrated a higher DA transporter specificity and higher level of non-specific uptake.

473.6

I-123 IBZM, a dopamine D2/D3 receptors radioligand, has been used for in vivo SPECT imaging to obtain measurements of dopamine receptor density. We have developed a simple kinetic analysis for time dependent IBZM uptake as an approximation of synaptic dopamine receptor density that enables comparison against a control radioligand association and presumably endogenous dopamine release. We tested this approach in normals and in two neurological conditions in which alterations of striatal dopamine activity are thought to occur: Parkinson's disease and Huntington's disease. In patients with asymmetric clinical signs of Parkinson's disease (PD) (n=13) there was a nonsignificant increase in mean basal ganglia/occipital ratio of IBZM activity at 2 hrs contralateral to maximal clinical signs. In patients with Tourette's Syndrome (TS) discordant for ratings of severity (n=6 twin pairs), there was no scan difference between twins in IBZM ratio. The rate of accumulation of IBZM (a parameter proportional to association and dissociation rates, receptor density and concentration of endogenous dopamine) in the basal ganglia over the 4 hr scan time revealed in the PD patients a mean slope that was significantly higher in the contralateral striatum (p<0.02). The more severely affected TS twins showed a significantly decreased slope when compared to the less affected twins (p<0.03) and in normal controls (p<0.03). We conclude that the rate of accumulation of striatal IBZM activity reflects synaptic dopamine activity and possibly dopaminergic release since it is increased in the setting of decreased striatal dopamine concentration (PD) and is decreased in a disease purported to have increased striatal dopamine activity (TS). This suggests that IBZM activity is a more sensitive indicator of synaptic dopamine activity than single time point measurements of striatal/occipital ratio.

473.7
NEUROCHEMISTRY REVEALS THE DEPLETION OF EXTRACELLULAR DOPAMINE IN THALAMIC NERVE ENDS IN THE RABBIT HYPOTHALAMUS. R. C. Moore, A. F. Moore, East Carolina Northport, DEpts. of Pediatrics and Neuroscience, University of Carolina, Chapel Hill, NC. 28630.

Withdrawal of chronic ethanol (EtOH) treatment in rats is associated with a profound inhibition of the mesolimbic dopamine (DA) system. DA depletion has been hypothesized to contribute to a neurotransmitter correlate of the dysphoric and depressive state associated with withdrawal. However the cellular mechanisms underlying this phenomenon have not been fully understood. Chronic EtOH treatment is known to be associated with an overactivity of L-type calcium channels. To assess whether L-type calcium channels have a role in the withdrawal-associated inhibition of the DA system, we studied the effect of the dihydropyridine calcium antagonist nimodipine on the extracellular DA in the ventral striatum of EtOH-withdrawn rats. In EtOH-dependent rats, twelve hrs following the last treatment (5g/kg every 6 hr for 6 days), dialyse DA levels were about 30% of control, sucrose-treated rats. Nimodipine (2.5 mg/kg s.c.) dose dependently reversed the fall in DA output toward control values. At the dose of 10 mg/kg nimodipine raised DA output to about 160% of control. In contrast, the drug had no effect in control rats. Thus, overactivation of L-type calcium channels can mediate the inhibition of the mesolimbic dopaminergic system during EtOH withdrawal, presumably through inhibitory influences to dopamine cells. In addition, our results suggest a potential role of nimodipine in the dysphoric and depressive state associated with EtOH abstinence syndrome.

473.9

Previous studies have shown that the genetic variability in the sensitivity to haloperidol-induced catalepsy is associated with the number of dopamine neurons in the substantia nigra zona compacta (SNZC) (JPET 266:431, 1995). To further investigate this relationship, we have used two methodologies. Neurleptic responsive (NR) and nonresponsive rats were selected from the new heterogenous stock/Northport (HS/Np). At S, the lines differed 6-fold in their sensitivity to haloperidol-induced catalepsy. Confirming previous results, tyrosine hydroxylase (TH) cell number was significantly higher in the NRN line; the difference was most pronounced in the rostral SNZC where TH cell number was increased 23% Forty-two C57BL/6J-D2A/2 (B6D2F2) F2 hybrids were phenotyped for haloperidol response prior to determining TH cell number. Paralleling the results in the selected lines, TH cell number was found to be proportional to the number of responsive animals. TH cell number was determined in 10 inbred mouse strains that were previously phenotyped for haloperidol response. Among the inbred strains no correlation was found between TH cell number and cell number. The reasons for the differences between the first two and third genetic strategy are not clear but may suggest that epistatic interactions are present in the HS and F2 strains but not the inbreds. This data brings into question the validity of using genetic correlations obtained from inbred strains.

473.10

We have previously demonstrated (JPET 61:341, 1992 and Psychopharmacol. 103:244, 1991) that NR and NRN lines selected from HS/1bg stock differ significantly in D1 receptor density. We found that there was a significantly higher somatodendritic dopamine receptor density throughout the midbrain but a lower receptor density in the lateral striatum. Recently, we confirmed that among 8 inbred mouse strains (C57BL/6J-D2A/2, C3H, BALB/c, LP, A, AKR and CBA), the strains least sensitive to haloperidol-induced catalepsy had the highest somatodendritic receptor density (LJPET 267: 538, 1993). However, differently than the selected lines, there was a trend to higher receptor density among the nonresponsive lines in the lateral striatum. These eight inbred strains were crossed to form a new HS stock. After 8 generations of random breeding, a new selection of HS and NRN lines was begun. At S, the lines differed 6 fold in their Emax for haloperidol-induced catalepsy. Receptor binding to the D1 family of receptors was determined using [1H]epibolide and quantitative receptor autoradiography. The data obtained parallel the results obtained from the inbred strains - receptor density was higher both in the substantia nigra and striatum. Similar results have also been obtained in C57BL/6J-D2A/2 F2 animals phenotyped for haloperidol response and D1 receptor density.

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473.11 MAPPING THE GENES CONTRIBUTING TO CHOLINERGIC AND DOPAMINERGIC NEURON DENSITY USING THE BDX/TY RI STRAINS. K. Denis*, B. Hitzemann and B. Hitzemann, Departments of Neurobiology and Behavior, and Psychiatry, State University of New York at Stony Brook, Stony Brook, NY 11794 and Psychiatry Service, VAMC, Northport, NY.

Two traits associated with neuroleptic-induced catecholysis are the density of cholinergic cells in the caudate-putamen and the density of dopaminergic cells in the substantia nigra (Hitzemann, et al., 1993). By analyzing the BDX/Ty series of mice we were able to obtain significant quantitative trait loci (QTL) associated with haloperidol-induced catecholysis (Kanes, et. al., 1994) and the above traits. These QTL include the microsatellites D8Mit22, D8Mit74 and D8Mit21 which map near the dopamine D3 receptor gene (Drd3). F2 hybrids generated from C57BL/6J and DBA/2J strains were used to confirm the associations between significant QTL by typing for haploider sensitivity and cholinergic and dopaminergic cell density, then genotyping for the significant QTL. This analysis has also revealed a significant QTL for cholinergic cell number at microsatellite D12Mit17, which is within proximity of the serotonin transporter gene. These markers are suitable candidates for positional cloning.

473.12 LESIONING THE MEDIAL PREFRONTAL CORTEX DECREASES MIDBRAIN DOPAMINE NEURON ACTIVITY. S.S. Shrestha, W.A. Shi and B.S. Bunn, Depts. of Psychiatry & Pharmacology, Yale Univ. New Haven, CT 06510.

Lesions in the prefrontal cortex (PFC) have been shown to profoundly affect subcortical dopamine (DA) transmission in animals, and this effect is believed to be mediated by cortical projections to DA terminal areas (see reviews by Grazi, J Neural Transm 91:111, 1993 and Deutch, J Neural Transm 91:197, 1993). However, since the PFC is also innervated by DA neurons located in the midbrain, lesions in the PFC may directly affect the electrical activity of DA neurons which should in turn influence DA release in terminal areas. To examine this possibility, we used extracellular single unit recording techniques to record DA neurons from both A10 and A9 in control and PFC lesioned rats. To lesion the medial PFC, we either made a unilateral stab to the mPFC or locally injected ibotenic acid into the mPFC. One hour after mechanical lesioning the number of spontaneously active DA neurons in both the A9 and A10 areas was decreased (A9: from 1.15±0.10 to 0.57±0.07/track, A10 from 1.41±0.09 to 0.77±0.09). Furthermore, the burst activity of A10 DA neurons was also reduced from 26.7±1.80 to 17.50±2.45. However, unlike the mechanical lesion, local injection of ibotenic acid only decreased the activity of A10 neurons. Thus, four to 20 days after the injection, the number of spontaneously active DA neurons in the A10 area was significantly reduced from 1.50±0.13 to 0.56±0.11 cells/track, whereas that in A9 area were not significantly affected. Surprisingly, the basal activity of DA neurons in the A9 area was increased (control: 3.65±0.28 spikes/sec, lesioned: 4.83±0.24 spikes/sec). These results suggest that lesions in the mPFC can significantly affect DA neuron activity located in the midbrain and this effect may contribute to the change in DA transmission in subcortical areas seen after PFC lesion. Supported by PHS award MH28849, the NPF, the NARSAD, and the State of Connecticut.

473.13 APOMORPHINE INCREASES EXTRACELLULAR GABA LEVELS IN THE PREFRONTAL CORTEX OF THE FREELY MOVING, CONSCIOUS RATS. A.C. Grobin* and A.Y. Deutch, Dept. of Psychiatry and Pharmacology, Yale Univ. Sch. Medicine, New Haven, CT 06510.

It has been hypothesized that dopamine (DA) in the prefrontal cortex (PFC) regulates GABA release function. DA axons form synapses with GABA neurones in the PFC. Furthermore, in vitro studies have reported that DA agonists will stimulate GABA release in the PFC. To test this hypothesis we used the DA D1 agonist apomorphine (APA) which was administered to male Sprague-Dawley rats and GABA levels determined in the PFC using in vivo microdialysis. APA was delivered directly to the PFC through the dialysis probe, or systemically through an indwelling subcutaneous catheter. GABA levels and relative amounts of amino acids in dialysates were determined using pre-column derivatization and high performance liquid chromatography (HPLC-EC). Local administration of APA (0.01 and 1.0 μM) produced a large increase in extracellular GABA. Systemic administration (0.5mg/kg) produced a pronounced increase in extracellular GABA of short duration; no increase in glycine was observed. Thus, both local and systemic administration of a DA receptor agonist increased extracellular GABA levels in the PFC. This report provides the first in vivo data to show that DA stimulates GABA release in the PFC. Thus, DA may inhibit pyramidal cell firing through stimulation of inhibitory GABA interneurons, as well as by directly synapsing on pyramidal cells. This arrangement has important implications for our understanding of the functional consequences of the loss of interneurons in the cortex of schizophrenics.

These studies were supported by MH-45124 and GM-07324.
473.17

The ventral pallium (VP) receives a dopamine (DA) input from the substantia nigra and ventral tegmental area. In locophoretic application of DA or the DA agonist SKF38393 alters the firing rate of many VP neurons (Napier and Maslowski-Cobuzio, Synapse, in press). Also, systemically administered DA, agonists increase the activity of many VP neurons (Maslowski and Napier, J. P. 200-103).

The present study investigated the effects of chronic DA depletion on the response of VP neurons to DA agonists and SKF38393. Rats were anesthetized with chloral hydrate and single neuron recording techniques were used to assess changes in sensitivity to microiontophoretic DA and SKF38393, and their effect on DA release. There was no change in the response of VP neurons to DA agonists. Consequently, it was suggested that DA agonists do not alter their “bad” firing rates. Instead, the response of VP neurons changes in a manner that is consistent with the observed behavioral changes.

473.18
WHY CAN’T HEMIPARKINSON RATS WALK NORMALLY? I. Q. Whishaw and E. I. Miklyaeva, Department of Psychology, University of Lethbridge, Lethbridge, AB, Canada, T1K3M4

The normal walking sequence of many tetrapods involves patterns of diagonal support in which one set of diagonal limbs supports weight while weight is unloaded from the others. We have examined walking in hemiparkinson rats (6-OHDA in the nigrostriatal bundle) using high speed video, cinemetic, and video support analysis. They use abnormal gait or turn ipsilaterally in tests of spontaneous locomotion rather than displaying the normal diagonal pattern of walking. An investigation of the underlying basis for this change showed that they are not able to use their “bad” limbs in shift to posture and contribute to the normal diagonal walking pattern. To compensate for this impairment they alter their movement patterns to place more reliance on their good limbs. Thus, it is suggested that hemiparkinson rats do not walk normally because they are relying upon two limbs rather than four to shift their weight.

473.19
WHY DO HEMIPARKINSON RATS TROT? E. I. Miklyaeva and I. Q. Whishaw, Department of Psychology, University of Lethbridge, Lethbridge, AB, Canada, T1K3M4

Hemiparkinson rats (6-OHDA in the nigrostriatal bundle) turn ipsilateral to their lesion spontaneously and under amphetamine and central stimulation of the nigrostriatal dopamine. They display sensorimotor abnormalities when eating, rearing, reaching and competing for food. Control rats move away from a “diagonal” posture supporting pattern, unloading the limb opposite to the lifted one and loading the other pair of limbs, when performing most behaviors. Hemiparkinson rats are not able to use their “bad” limbs in this way. Consequently they modify their posture and movement to rely on the ipsilateral “good” limbs to actively adjust support. This change biases their behavior such that they display a variety of anomalous movements that include ipsiversive rotation spontaneously and under amphetamine, backward dodging when they protect their food from rodents, and atypical standing to reach for food. Apomorphine restores more normal movements. The results show that following DA depletions the new supporting patterns are related to the many changes in behavior typical of hemiparkinson rats. Thus, it is suggested that they turn because they have to.

472.21
PRIOR EXPOSURE TO INTRA-RIA AMPHETAMINE ENHANCES THE INDUCTION OF IMMEDIATE-EARLY GENE EXPRESSION IN THE RAT FOREBRAIN BY SYSTEMIC AMPHETAMINE. P. Vesin* and G. S. Robertson, Dept. of Psychiatry, University of Chicago, Chicago, IL 60637 and Department of Pharmacology, University of Ottawa, Ottawa, Canada K1H 8M5.

Injections of amphetamine into the ventral tegmental area (VTA) have been shown to enhance the locomotion and increase the extracellular DA concentrations (N.Acc.) dopamine (DA) produced by a subsequent systemic amphetamine challenge. The present experiment investigated whether prior exposure to such injection would also affect the subsequent induction by systemic amphetamine of immediate-early gene activation in the N.Acc., the antero-medial striatum and the medial prefrontal cortex, forebrain projection fields of A10 DA perikarya in the VTA were administered injections of either d-amphetamine (2.5 µg/0.5 µl/side) or saline into the VTA, one injection every third day. One week following the last injection, animals were challenged with amphetamine (10 mg/kg). Results indicated that responses to repeated injections of DA were increased (generally two fold) expression of FOS, FOS-B, JUN-B and NGFI-A. Animals exposed to VTA amphetamine showed increased (generally two fold) expression of FOS, FOS-B and JUN-B in all A10 projection fields when compared to VTA saline preexposed animals. The two groups did not differ in amphetamine induced expression of NGFI-A in any area. This enhanced induction by amphetamine of immediate-early genes of the fos/jun (leucine-zipper) family in VTA amphetamine preexposed animals is consistent with the sensitized DArgic response to this drug reported in the literature. This enhanced amphetamine induced expression of DArgic genes (N.Acc.) while NGFI-A (of the zinc finger immediate-early gene family) is induced by amphetamine, it does not appear to be involved in the differential expression of the behavioral and DArgic responses to amphetamine by these two groups.

473.22
Changes in cochlear tyrosine hydroxylase immunoreactivity after repeated exposure to cocaine. B.G. Shigemura, S.Y. Liu, D.Z. Piotrowski, E.P. Schoener*, Dept. of Otolaryngology, Henry Ford Hospital, Detroit, MI 48202.

The site of action of cocaine in the auditory system may include structures peripheral to the ventral cochlear nucleus, including the cochlea. Previous studies in our laboratory examined the acute and chronic effects of cocaine on cochlear function. Experiments designed to study acute effects of cocaine on cochlea demonstrated a significant decrease in both the amplitude of the compound action potential of the auditory nerve (N1) and cochlear blood flow. On the other hand, experiments designed to study chronic effects of cocaine on the cochlea show an enhancement of the N1 amplitude of the auditory nerve.

In order to understand the underlying mechanisms involved in chronic cocaine action in the cochlea, this experiment was designed to examine the effects of repeated administration of cocaine on the cochlear cochanneling systems in chinchilla using immunohistochemical techniques. Twelve chinchillas were randomly assigned to either control or experimental groups. Animals in control group were administered saline while animals in experimental group were administered 15 mg/kg cocaine IP daily for 42 days. Animals were sacrificed 24 h after the last treatment. The cochleacochleal system was evaluated using a polyclonal antibody to tyrosine hydroxylase (TH). Microscopic examination of whole-mounted tissue revealed TH immunoreactive fibers in the outer spiral bundles. This change was described as a robust increase in the number of fibers in the outer spiral bundles. Total number of fibers per cochlea between the two groups demonstrated a significant decrease in cocaine-treated animals. (Supported in part by NIDA/NSH grant DA07554)

Whether tetrodotoxin-resistant dopaminergic (TIDA) neurons contain dopamine (DA) autoreceptor has been a controversial issue. Earlier study from this lab using single-unit recording of dorsomedial/ventrolateral arcuea (ARC) neurons in rat brain slices found that DA inhibited 37% of ARC neurons recorded. Recently, immunohistochemical studies, however, reveal that DA neurons are localized in the dorsomedial part of the ARC only. By focusing our recording in that region, we found that DA in 50-250 nmol range now inhibited a significant number of ARC neurons tested (74.2% of 182 units). Apomorphine, a DA agonist, had no effect (38.1% of 21 units). Cocaine, an abusive drug whose effect is believed to increase DA concentration in synaptic clefts by inhibiting DA reuptake, also inhibited a significant number of ARC neurons by itself (51.5% of 97 units), but with a lesser efficacy. Cocaine co-administered with DA, however, potentiated the inhibitory effect of DA in 82% of DA-responsive units (n=39). The results demonstrate clearly that DA indeed had a predominantly inhibitory effect on presumed DA neurons in the dorsomedial ARC. The effects of cocaine, either by itself or with DA, further support this notion.


The 5-HT3 receptor mRNA has recently been shown to be expressed as two forms (5-HT3α1 and 5-HT3α3) varying by 18 base pairs in the third intracellular loop (Hope et al., Eur. J. Pharmacol., 245:187, 1993). As the short form of the 5-HT3 receptor lacks a cassekinase II phosphorylation site in the region corresponding to the alternative splicing, the question of the functional roles of the two variants has been raised. We have studied the regulation of the expression of mRNAs coding for the two variants in a variety of cells using different culture conditions. Relative abundance of the two mRNAs was determined by quantitative PCR over the linear range of the assay by using primers flanking the site of alternative splicing. Reverse transcribed mRNAs for both forms (L+ and L−) were performed by competitive PCR using as an internal standard a deleted DNA corresponding to a 460 base pair fragment starting at transeptumbrine domain-2 and ending at TM-6. Differentiation significantly reduced the abundance of the overall mRNA coding for 5-HT3 receptors in different proportions depending on the cell type (dibutyryl cAMP, with or without TPA, theophyllin + PGE1...). In addition, the relative proportions of the two splice variants changed significantly when compared with control cells, since the ratio S/L increased with exposure time to the differentiating agent (Control: 2.75; DR-AMP 1 mM, 1 day: 3.76, 2 days: 4.29, 3 days: 4.43, 5 days: 5.36, 7 days: 6.42). As functional changes have already been described for 5-HT3 receptors upon differentiation in these cells (Shao et al., J. Neurophysiol. 65:650, 1991), our results suggest different functional roles for 5-HT3α1 and 5-HT3α3.


Based on the predicted amino acid sequence of the cloned rat 5-HT3 receptor cDNA (Sugita et al., Soc. Neurosci. Abstr., 19:632), we designed seven different peptides of 15-19 residues that were expected to be antigenic were conjugated to bovine serum albumin and used as immunogen to develop anti-5-HT3 receptor antibodies in New Zealand White rabbits. The sera obtained after 3-H injections were specific for the anti-serum terminal, extracellular region, and four from the large intracellular loop, between transmembrane domains 3 and 4 (M1) of the putative pore region. Antibody production was evaluated by solid-phase ELISA and immunoblot analysis. Peptides corresponding to the extracellular region of the protein gave antibody of very low avidity. Two of the M1 peptides (P1 and P2) corresponding to the large intracellular loop produced antisera of high titer. These antisera when tested on a dot blot analyses were able to detect 0.2 ng of synthetic peptide at dilution of 1:2000.

Membranes from brain and NG-108-15 cells were solubilized with Triton X-100. By western blots, and 5-HT3-R antibodies recognized a single band (molecular weight close to 5-HT3-R) in the detergent soluble fractions. Western blot and immunoblot analysis was abolished when antibodies were preadsorbed with the corresponding peptide (P1). Western blot analysis revealed 9 different bands and shapes on rat brain tissue: small round cells in the striatum; medium bipolal and multipolar cells in cortical areas and hippocampus. Immunoblotted neurons were also found in the olfactory bulb, olfactory tubercle and amygdala. The pattern of distribution of the immunoblotted cells is in agreement with that reported for the localization of 5-HT3-R transcripts (Johnsen and Heinemann, Soc. Neurosci. Abstr., 19:632). Our data indicate that 5-HT3R immunolabeled neurons are prominent in areas associated with the DA mesolimbic system. (Supported by AA 06420).

In contrast to studies indicating that applications of 5-HT\textsubscript{1A} receptor agonists can fast a depolarizing action, which is rapidly desensitized, on peripheral or cultured cells, we have found that 2-methyl-5-HT elicits a slow depresor action with no desensitization on cells in the medial prefrontal cortical (mPFC). In addition, 5-HT\textsubscript{1A}-like receptors in the mPFC might be coupled to the phosphoinositide (PI) hydrolysis. To further characterize the pharmacological properties of 5-HT\textsubscript{1A} receptors in the mPFC, using the technique of single cell recording and interneuronal responses, we have compared the depressant action of various 5-HT\textsubscript{1A} receptor agonists on the firing of mPFC cells. The rank order of effectiveness was: 5-HT > 5-HT\textsubscript{1A} > 5-HT\textsubscript{1B} = 2-methyl-5-HT = mCPBG (1-(1-methylpiperidino)-benzamine) = PBB = PCPBG = PCBPG. Furthermore, in the mPFC, d-tubocurarine not only blocked the depressant action of 5-HT\textsubscript{1A} receptor agonists but also antagonized that of d-amphetamine and GABA. In fact, d-tubocurarine alone accelerated the firing of mPFC cells. In another research paradigm, the effect of various 5-HT\textsubscript{1A} receptor agonists on PI turnover were investigated and compared using the tracer technique of this mPFC. The rank order of potency was: 5-HT > 5-HT\textsubscript{1A} > 2-methyl-5-HT > mCPBG = PBB = PCPBG = PCBPG. In summary, following the two new findings further indicate that 5-HT\textsubscript{1A}-like receptors in the mPFC are pharmacologically different from those in the penta
tetrapyrrole (pTP) or 1 in the mPFC, mCPBG is not the most potent 5-HT\textsubscript{1A} receptor agonist, and 2-d-tubocurarine is not a specific antagonist for 5-HT\textsubscript{1A}-like receptors.

**EFFECT OF GRANISTIM ON ACUTE AND DELAYED CISPLATIN-INDUCED EMESIS IN THE PIGLET.** L. Groleau, S. Milano, P. Blower \& D. Romain. URA CNRS 8332, St. Jerome, 13397 Marseille Cedex 20, France; SmithKline Beecham Pharmaceuticals, UK (1) and France (2).

Emesis in cancer chemotherapy is classified as acute or delayed when occurring during or after the 24 hr following systemic treatment, respectively. It remains unclear whether serotonin 5-HT\textsubscript{3} receptors are involved in delayed emesis. In 19 weaned piglets, granisetron was tested on both acute and delayed emesis. Vomiting was induced by an iv injection of cisplatin (5.5mg/kg) and animals were observed for 60 hr. In group 1, 12 piglets received an initial (15 min prior to cisplatin) single dose (5mg/kg, n=5); 1mg/kg, n=3; 3mg/kg, n=3 or 7mg/kg, n=4) of granisetron. In group 2, 7 piglets received in addition to the initial dose (0.25mg/kg, n=2; 1mg/kg, n=5), a supplementary injection (same dose as first) each 5 hr during the 30 hr period. In group 1, single doses of granisetron prevented vomiting during the first 5-18 hr in a dose dependent manner. All animals exhibited a delay in the onset of vomiting, the time to the first vomiting was (13.5±8.8) hr for those which was higher than those of animals not treated with granisetron (9.2±8.6). In group 2, repetitive administration of granisetron was beneficial in reducing both the acute and delayed phases of vomiting. Indeed, 6/7 piglets (0.25mg/kg, n=1; 1mg/kg, n=5) during the acute phase and 4/7 (1mg/kg, n=4) during the delayed phase did not vomit. These results suggest that serotonin 5-HT\textsubscript{3} receptors might be involved in the development of delayed emesis in this species.

**THE PIGLET: AN ANIMAL MODEL FOR STUDYING CISPLATIN-INDUCED DELAYED EMESIS.** S. Milano, L. Groleau, P. Blower \& D. Romain. URA CNRS 8332, St Jerome, 13397 Marseille Cedex 20, France; SmithKline Beecham Pharmaceuticals, UK (1) and France (2).

The pathogenesis of cisplatin-induced delayed emesis being obscure, we investigated the suitability of the piglet as a model for studying delayed emesis. The piglet was implanted surgically with a stainless steel cannula in the jugular vein and electrodes for recording ECG and abdominal EMG. After a 4-5 days recovery, piglets were hydrated (iv, saliné 4% body weight, 100ml/kg/hr) and then given cisplatin (5.5mg/kg, iv). Recordings were made continuously for the following 48 hr. Animals were not fed but received continuous iv infusions of glucose and polyionic solutions. All piglets responded with both acute and delayed emesis. In the acute phase of emesis, the first vomiting occurred with a latency of 1:31±4.3 min after cisplatin administration. Emetic intensity reached a peak (5 vomits/hr) within 120 min and then decreased rapidly, so that no vomiting were observed between the 15th and 16th hr. The mean number of vomiting during the first 16 hr was 18.6±2.6. Delayed emesis started at the 17th hr and was observed to last until the 58th hr. Emetic intensity of the delayed phase reached a peak (0.8 vomits/hr between the 19th and 21st hr and the mean number of vomiting during the whole of the delayed phase was 9.2±2.6. Cisplatin inducing delayed vomiting in the piglet with both a weak toxicity and a relatively low emetic potential is a suitable model in which to study the pathogenesis of delayed emesis.

**AGONIST POTENCY AND AFFINITY AT 5-HT\textsubscript{1A} RECEPTORS EXPRESSED IN CHINESE HAMSTER OVARY (CHO) CELLS.** J.G. Heniez, K.D. Duria, L.E. Gooden and C.O. Berti. Dept. Pharm Biol. Texas Health Sc. Center, San Antonio, TX 78284; Dept. of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA. 19104.

The relationship between agonist potency and affinity was investigated in CHO cells stably expressing the 5-HT\textsubscript{1A} receptor (CHO-5-HT\textsubscript{1A} cells). In membranes from CHO-5-HT\textsubscript{1A} cells, the specific binding of the radiolabeled 5-HT\textsubscript{1A} receptor agonist [\textsuperscript{3}H]8-OH-DPAT (1-12 nM) was inhibited essentially completely (97±1.5%) by GppNHp [EC\textsubscript{50}= 2.8 ± 0.3 µM]. Saturation experiments using membranes from CHO-5-HT\textsubscript{1A} cells indicated that the binding of [\textsuperscript{3}H]8-OH-DPAT (0.05-50 nM) in the absence of guanine nucleotides, was to a single site (Kd = 1.1 nM, Bmax = 1 pmol/mg protein). Taken together, these data suggest that in CHO-5-HT\textsubscript{1A} cells all of the 5-HT\textsubscript{1A} receptors are coupled to G proteins and exist in a high affinity state as determined by radioactive agonist binding. To determine whether agonist potency can be related to the high affinity state of the receptor, EC\textsubscript{50} values of agonists to inhibit forskolin-stimulated [\textsuperscript{3}H]cAMP accumulation in CHO-5-HT\textsubscript{1A} cells were compared with their Ki values for the inhibition of [\textsuperscript{3}H]DPAT binding. The EC\textsubscript{50} values to the EC\textsubscript{50} values were near unity: 8-OH-DPAT, 1.40 ± 0.09 nM; 5-HT, 0.24/2 ± nM; gepirone, 0.557 ± nM; gepirone, 0.557 ± nM. Thus in CHO-5-HT\textsubscript{1A} cells, the potency of agonists appears to be related to their affinity at the high affinity state of the receptor. Supported by USPHS grants MH46125 and MH52399.
SEROTONIN RECEPTORS: EFFECTOR MECHANISMS

475.3
5-HT<sub>2</sub> AND 5-HT<sub>3</sub> RECEPTORS ARE RESPONSIBLE FOR THE HYPNOTIC EFFECT OF SEROTONIN IN A HUMAN TUMOR OF NEUROCHROMAFFIN ORIGIN
Lucia A. Filionti*1, F. P. Volinalli and R.G. Cattaneo
Department of Pharmacology, University of Milan, Milan
Small cell lung carcinomas (SCLC), as aggressive human tumors often associated with tobacco smoking, possesses neuroendocrine features. These include the presence of neuroendocrine granules containing hormones and neuropeptides and the expression on their surface of specific receptors and voltage-dependent Ca<sup>2+</sup> channels. We have found that serotonin (5-HT) is contained, and is autocrine in the two SCLC cell lines GC1 and NCI-H60. The autocrine effect of 5-HT is not counteracted by ketanserin, D5L 205-350 and 311-408 which are antagonists of the 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> receptors, respectively. We found that the 5-HT<sub>2</sub> agonist mirtazapine and the 5-HT<sub>3</sub> agonist O-29-DOP is capable of increasing DNA synthesis in the SCLC cell lines, with lower efficacy but higher potency than 5-HT (EC<sub>50</sub> 25 nM and 90 nM respectively). Their effects on DNA synthesis were completely additive and together they reached the maximal effect elicited by 5-HT alone. Speromine (1 μM) and the 5-HT<sub>3</sub> antagonist 5HT 216-252 (1 μM) inhibited the 5-HT autocrine response by 60%.
When cells were cultivated at higher density, the two 5-HT<sub>3</sub> antagonists were able to inhibit basal cell proliferation. Taken together, our results suggest that 5-HT should be added to the list of autocrine growth factors for SCLC cells. The autocrine effect of 5-HT is due to stimulation of both 5-HT<sub>2</sub> and 5-HT<sub>3</sub> receptor types. The design of specific antagonists might be useful for the growth control of human small cell lung carcinomas.

475.4
CONSTITUTIVELY ACTIVE SEROTONIN 2C (5HT<sub>2C</sub>) RECEPTORS STIMULATE DNA REPLICATION IN A TRANSFECTED CELL LINE
R.S. Westphal and S. Sander-Bush*2
Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232
Agonist activation of the serotonin 2C (5HT<sub>2C</sub>) receptor expressed in NIH 3T3 fibroblasts has been shown to result in the development of a transformed phenotype. In light of recent evidence demonstrating 5HT<sub>2C</sub> receptor constitutive activity, the contribution of agonist independent receptor activation to transformation of NIH 3T3 fibroblasts transfected with 5HT<sub>2C</sub> receptor cDNA was examined. [3H]thymidine incorporation was used as a measure of cell growth in serum starved NIH 3T3 fibroblasts transfected with 5HT<sub>2C</sub> receptor cDNA. Three classes of 5HT<sub>2C</sub> receptor ligands were distinguished. In transfected, but not nontransfected, fibroblasts basal levels of [3H]thymidine incorporation were increased by agonists, decreased by inverse agonists and minimally affected by neutral antagonists. The effects of agonists and inverse agonists were blocked by a neutral antagonist. Pharmacological characterization of agonist-mediated increases in [3H]thymidine incorporation was consistent with properties of the 5HT<sub>2C</sub> receptor. In addition, the rank order of potency of inverse agonists to decrease basal [3H]thymidine incorporation was reciprocally modulated by 5HT<sub>2C</sub> receptor agonists and inverse agonists. These data suggest that constitutively active 5HT<sub>2C</sub> receptors stimulate cell division and may contribute to the development of a transformed phenotype (Supported by the National Institute of Mental Health, MH 30407).

475.5
5-HT<sub>2C</sub> RECEPTOR ACTIVATION INCREASES ARACHIDONIC ACID RELEASE: W.P. Clarke*1, K. A. Berg*2 and S. Mayanagi*3
Departments of Pharmacology1 and Anesthesiology, Mount Sinai School of Medicine, CUNY, New York, NY 10012
The 5-HT<sub>2C</sub> (formerly 5-HT<sub>2A</sub>) receptor is a member of the 5-HT receptor family which have been shown to be coupled to phospholipase C (PLC)-mediated phosphatidylinositol (PI) lipid hydrolysis. Recently it has been shown that some PLC-coupled receptors can also activate phospholipase A2 (PLA<sub>2</sub>) to liberate arachidonic acid (AA). We have investigated the effects of 5-HT<sub>2C</sub>-receptor agonists on the PLC-PLA<sub>2</sub>-AA pathway. CHO cells were transfected with human 5-HT<sub>2C</sub> cDNA and a stable line expressing 300,000 protein was selected for study. Cells were incubated for 4 h at 37°C with 0.1 μM of [1<sup>4</sup>C]-AA. After washing, 5-HT<sub>2C</sub>-agonist-induced release of [1<sup>4</sup>C]-AA was measured at various time points (3-50 min). 5-HT (EC<sub>50</sub> 28 nM), DOPA (EC<sub>50</sub> 100 nM) and quipazine (EC<sub>50</sub> 1.2 μM) each increased AA release by 43% and 32%, respectively. The EC<sub>50</sub> values for each of these agonists for PI hydrolysis were similar to those for AA release (26 ± 13 nM and 1.5 μM for 5-HT, DOPA and quipazine, respectively). 5-HT<sub>2C</sub>-mediated AA release desensitized by 60% after 15 minutes treatment with 10μM 5-HT, whereas ATP-mediated AA release was unaffected. 5-HT<sub>2C</sub>-mediated AA release was insensitive to treatment with pertussis toxin. Both 5-HT and medium-induced AA release was reduced (90%) in the absence of extracellular Ca<sup>2+</sup>. Inhibitors of PLC (U-73122 and ET-18-OCH<sub>3</sub>), at concentrations that reduced PI hydrolysis by greater than 90%, had little or no effect on 5-HT<sub>2C</sub>-agonist mediated AA release. However the PLA<sub>2</sub>-inhibitor nap cyancine completely blocked 5-HT<sub>2C</sub>-mediated AA release but did not affect 5-HT-mediated PI hydrolysis. These results suggest that 5-HT<sub>2C</sub>-receptors couple to the PLA-2 AA pathway in CHO cells. (Supported by UPHS grants GM 34825, DA06620, MH48125, HD26437).

475.6
5-HT<sub>1B</sub>/1D receptor function via arachidonic acid metabolism. K. A. Berg1, S. Mayanagi2, and W. P. Clarke1
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5-HT<sub>1B</sub>/1D receptor-mediated inhibition of forskolin-stimulated Ca<sup>2+</sup> accumulation (FSAC) is inhibited by activation of transfected human 5-HT<sub>2C</sub> but not 5-HT<sub>2A</sub>, receptors in CHO cells (Berg et al., Mol. Pharmacol., in press). We have investigated the role of phospholipase A2 (PLA<sub>2</sub>)-mediated arachidonic acid (AA) metabolism as a mediator of the effect of 5-HT<sub>2C</sub> receptor activation.
In CHO cells stably expressing 5-HT<sub>2C</sub>-receptors (200 fmol/mg protein), 5-HT<sub>2C</sub>-agonists (such as DO1) increase AA release via PLA<sub>2</sub>-dependent mechanisms (see Clarke et al., this meeting). As reported previously, activation of 5-HT<sub>2C</sub>-receptors with DOI (1 μM) abolished 5-carboxamidotryptamine (5-CT, 5-mg/ml)-induced inhibition of FSAC. This effect of DOI was blocked following incubation with the PLA<sub>2</sub> inhibitor napycine (100 μM). The inhibition of FSAC (mean ± SE, n=5) was as follows: 55 ± 5% for 5-CT alone vs. 9 ± 7% for 5-CT + DOI, in the presence of napycine, 58 ± 6% for 5-CT alone vs. 61 ± 8% for 5-CT + DOI. Furthermore, the cyclooxygenase inhibitor indomethacin (2 μM) also blocked the DOI-mediated reduction of 5-CT inhibition of FSAC (73%±3% vs. 68%±4% for 5-CT and 5-CT + DOI respectively, n=5). Activation of PLA<sub>2</sub> by melittin (2.5 μg/ml) as well as activation of naturally expressed ATP-receptors abolished 5-CT-mediated inhibition of FSAC and napycine blocked these effects. These data suggest that PLA<sub>2</sub>-AA release mediates the 5-HT<sub>2C</sub>-receptor regulation of the 5-HT<sub>1B</sub>/1D receptor pathway in CHO cells. (Supported by UPHS grants GM 34825, DA06620, MH48125, HD26437).
PROTEIN KINASE C (PKC) INHIBITORS ENHANCE 5-HT ACTIVATION OF INTERNEURONS IN RAT PERFORM CORTEX MEDIATED BY THE 5-HT5A RECEPTOR. D. L. Mares and G. K. Azghani. Dept. of Psychiatry and Pharmacology, Yale School of Medicine, New Haven, CT 06520-

The excitation of interneurons in rat perform cortex is known to be mediated via stimulation of 5-HT5A receptors. Since activation of the phosphodiesterase (PDE) second messenger by 5-HT acting on 5-HT2A receptors leads to increased intracellular calcium, we explored the role of PKC in the 5-HT5A signal transduction mechanism by testing structurally distinct PKC inhibitors. Experiments were performed on interneurons isolated by the negative selection of layers II and III in periform cortex slices. A specific PKC inhibitor, the benzolothiazidone alkaloid dithienyromethane, increased the excitation of interneurons by 5-HT (100 nM). The isoquinolinesulfonamide kinase inhibitors H7 and HA 1004 (100 nM) were also tested. H7 application robustly enhanced the excitation of interneurons by 5-HT. In contrast, H8 application only weakly enhanced the excitation of interneurons by 5-HT (100 nM) and HA 1004 had no effect. None of these PKC inhibitors altered the excitation of interneurons by NE (100 nM). The rank order potency of these isoquinolinesulfonamides is in agreement with the rank order potency of these compounds for inhibition of PKC, but not for PDE, PKG, or PLC.

Based on these results we propose that: 1) The activation of PKC by 5-HT rapidly desensitizes the 5-HT5A-mediated excitation; 2) PKC inhibitors prevent this rapid desensitization, unmasking the effect of the 5-HT5A receptor response. Thus, activation of PKC, rather than mediating the excitation action of 5-HT at the 5-HT5A receptor, appears to have a negative feedback role.

SEROTONIN RECEPTORS: MOLECULAR BIOLOGY

SITE-DIRECTED MUTAGENESIS OF THE 5-HT3 RECEPTOR: IDENTIFICATION OF RESIDUES INVOLVED IN GATING AND BLOCKING RECEPTOR ACTIVATION. N. J. Stocks, P. V. Vardy, P. Colledge, J. A. Bignall, P. G. Rose, and B. G. Dover. Yale University School of Medicine, New Haven, CT 06510.

Throughout the central and peripheral nervous system multiple 5-HT receptor subtypes are expressed to the superfamily of G protein-coupled receptors.

The molecular processes by which agonists and antagonists bind to 5-HT receptors are currently unknown.

In order to investigate the molecular basis for ligand binding to the 5-HT3 receptor we have constructed a site-directed mutagenesis model for the receptor using the atomic coordinates of the structurally related bacteriorhodopsin as a template. Based on our model and data from other studies we have chosen six residues, proposed to be involved in 5-HT binding, for site-directed mutagenesis studies. First, we investigated the role of two aromatic residues (Phe328 and Phe329) in transmembrane domain IV, that are conserved among all G protein-coupled 5-HT receptors. To investigate the role of these aromatic residues in ligand binding and receptor activation we have substituted these residues for alanine. Additional site-directed mutations were performed for two serine residues in transmembrane domain IV and six serine residues in transmembrane domain V. Both sites in transmembrane domain IV are conserved among all members of the 5-HT receptor family, with the exception of the 5-HT1A receptor in which Ser 187 is replaced by a Gly, and in the recently cloned 5-HT2F receptor in which this residue is replaced by an Ala. The Ser in transmembrane domain V is present in all members of the 5-HT5A-like (5-HT2A, 5-HT2C, 5-HT1B) receptors but not in 5-HT1D-like receptors. In our model, these three Ser residues are in a position that they may interact with 5-HT. Finally, we investigated the role of an acidic residue in transmembrane domain IV, which is conserved only in cationic amine receptors and which is postulated to interact with the positively charged amine group of 5-HT. Mutations and wild-type receptors have stably been transfected into NG108 cells and are currently pharmacologically characterized. Initial experiments indicate that some of the mutations affect differentially agonist binding as compared to antagonist binding.
476.3 AN AROMATIC RESIDUE AT POSITION 340 IS ESSENTIAL FOR ERGOT BUT NOT ERGOPEPTIDE BINDING TO THE 5-HT2A RECEPTOR. MS Choudhary**, A.Ulner, RB Westkaerper, RA Glennon, and BL Roth*. Department of Psychiatry, Case Western Reserve University Medical School, Cleveland, OH.

We have investigated the molecular requirements for drug binding to serotonin2a (5-HT2a) receptors and previously discovered a single, highly conserved, aromatic residue, Phe340, which is essential for ergot binding to the 5-HT2a receptor. To clarify the details of ergot- receptor interactions, an additional 8 mutations were constructed and expressed in COS-7 cells. The effects of these point mutations on the binding of 14 different ergot and ergopeptide derivatives were then determined. Mutations at position 340 in which the aromatic residue was substituted for an aromatic residue (e.g. F340L, F340A) greatly diminished ergot (100-1000 fold) decrease in Kd) but not ergopeptide (<10 fold decrease in Kd) affinities. By contrast, mutations at position 340 in which an aromatic residue was conserved (e.g. F340Y) did not affectably affect or ergopeptide affinities. Mutations at position 339, in general, had either no effect (F339A, F339L) or modest effects (F339V) on ergot and ergopeptide Kd's. The F125 mutations had little effect on ergot and ergopeptide binding. Two molecular models (one in which ergots and ergopeptides bind to different residues, another in which they utilize similar residues) were evaluated using three-dimensional computer-graphic models of 5-HT2A receptor-ligand interactions. The results suggest that an aromatic residue at position 340, but not 339, is essential for ergot but not ergopeptide binding to the 5-HT2A receptor.

476.4 A SMALL REGION IN THE SIXTH AND SEVENTH TRANSMEMBRANE DOMAINS OF THE HUMAN 5-HT1D* AND 5-HT1E* RECEPTORS DETERMINES 5-CARBOXAMIDOTRYPTAMINE AFFINITY. E.M. Parker*, D.A. Shapiro, and R.A. Gilbert. Departments of Psychobiological and Molecular, Chemistry, and Biological Sciences, California Institute of Technology, and Departments of Psychiatry, Case Western Reserve University, Cleveland, OH.

We have shown that the human 5-HT1D* receptor has high affinity for 5-c-arboxamidotryptamine (5-CAT) whereas the 5-HT1E* receptor has low affinity for this compound. In order to ascertain the structural basis for this difference, a series of chimeric 5-HT1D*5-HT1E* receptors was constructed. Using radioligand binding experiments showed that a stretch of 57 amino acids extending from the beginning of the sixth to the middle of the seventh transmembrane domain of these two 5-HT receptors was largely responsible for determining 5-CAT affinity. All the chimeric receptors had high affinity for 5-HT and yohimbine. Supporting the results of this study, the gross three dimensional structure of the chimeric receptors was not altered. A mutant 5-HT1D* receptor in which two residues in the sixth transmembrane domain were changed to the corresponding residues in the 5-HT1E* receptor (E333G334 converted to K333G334) showed markedly lower affinity for 5-CAT than did the wild type 5-HT1D* receptor but retained high affinity for 5-HT and yohimbine. Neither single mutation alone (i.e. E333 converted to K333 or G334 converted to E334) was sufficient to alter 5-CAT affinity. These data suggest that two amino acids in the sixth transmembrane domain largely determine the affinity of the 5-HT1D* and 5-HT1E* receptors for 5-CAT.


Serotonin, a modulatory neurotransmitter, acts on different receptors to elicit a variety of post synaptic responses. In particular, serotonin can elicit short-term and long-term facilitation of the monosynaptic connections between the sensory and motor neurons of the Aplysia gill-withdrawal reflex. The short-term actions of 5-HT likely involve activation of at least two types of 5-HT receptors, one coupled to cAMP and PKA and those coupled to PKC. To analyze this mechanism on the molecular level, we isolated two 5-HT receptor genes from Aplysia, Ap5HT2A and Ap5HT2B and one encoding an octopamine receptor, ApOctR. Both 5-HT receptors contain characteristic seven hydrophobic regions. The amino acid sequence in this putative transmembrane region is homologous to those of other serotonin receptors. The protein homology between the two receptors is 80%. Homology with mammalian 5-HT1A, 5-HT1C, 5-HT2, 5-HT3 receptors Drasaphila 5-HT receptor Drs1, Drs2b, Lynemaus 5-HT receptor Lym1, are 37%, 39%, 41%, 38%, 35% and 32% respectively. When expressed in mammalian cells both Ap5HT2A and Ap5HT2B stimulate phosphoinositol turnover by phospholipase C activation and mobilize intracellular Ca++ . Both are expressed in the Aplysia CNS. To further analyze the role of CAMP we also isolated an octopamine receptor that stimulates adenyl cyclase to increase cAMP over 500-fold in mammalian cultured cells. Since the sensory neurons of Aplysia lack octopamine receptors, transfection of this receptor into the sensory neurons should allow us to study the short- and long-term consequences of the selective activation of the CAMP pathway.


We isolated a mouse brain cDNA library and a human genomic library the genes encoding the 5-HT5A and 5-HT5B serotonin receptors. Amino acid sequence comparisons revealed that the human 5-HT5A receptor is 85% homologous to the mouse 5-HT5A receptor. We only found one human 5-HT5B pseudogene raising the possibility that there is no functional homolog of the HSA receptor. The mouse 5-HT5A receptor was expressed in Cos-7 cells, the mouse 5-HT5A receptor displayed (Kd=0.84 nM) and low affinity (Kd=13 nM) binding sites for the radiolabelled ligands [3H]10B-222 and [3H]10B-223. Both sites were insensitive to GTPyNop suggesting that the 5-HT5A receptor is not coupled to G protons in Cos-7 cells. The pharmacological profile of the 5-HT5A receptor is the profile of a G protein, which was altered on binding PKNop in Cos-7 and NIH 3T3 cells expressing the receptor. We did not detect any change in adenylate cyclase or phospholipase activity in response to serotonin. We are currently analyzing the possible coupling of 5-HT5A receptors to ion channels in a variety of neuronal cell lines. In order to study the function of the 5-HT5A receptor we are using gene targeting techniques to generate transgenic mice lacking this receptor.


The 5-HT6 receptor, one of three known subtypes of mammalian G-coupled serotonergic receptors, has garnered interest for its high affinity for the atypical neuroleptic clozapine. We previously reported the cloning of the rat 5-HT6 receptor and now report the isolation of the corresponding human gene and its chromosomal localization.

The human 5-HT6 receptor is a 440 amino acid polypeptide that is 96% identical to its rat homologue within transmembrane regions. The nucleic acid sequences of the open reading frames from the two species are approximately 84% identical. Comparison of genomic and cDNA sequences reveals that the 5-HT6 gene is approximately 3 kb intron occurs after base 714 and a 190 bp intron occurs after base 873. Using hybridization to a somatic cell hybrid panel, the 5-HT6 receptor gene was localized to 1p35-pter. This localization indicates that the 5-HT6 and the 5-HT1D loci may be closely linked. Such clustering has not been previously found within the serotonin receptor family. We have tentatively identified an 1p35-pter polymorphism within a 5-HT6 coding region, which may be informative in linkage studies examining the role of this receptor in various human diseases.
**477.2**

**SEROTONIN IA RECEPTOR BINDING IN THE ENTORHINAL CORTEX IN SCHIZOPHRENIA AND SUICIDE.** B.C. Ohlman, H. M. Hermann, C.E. Squires, D.J. Saboisa* and J.E. Kleinman.

Clinical Brain Disorders Branch, NIMH, Neuroscience Center at St. Elizabeth's Hospital, 2700 M.L. King Ave S.E., Washington, DC 20032.

Over the years, there has been a growing interest in the role of the entorhinal cortex in various neuropsychiatric disorders, such as Alzheimer's disease and schizophrenia. In this study we examined 5HT1A receptor density in post-mortem entorhinal cortex, using quantitative autoradiography comparing patients with schizophrenia to normal controls and non-schizophrenic neuroleptic-treated psychiatric patients. Blocked sections of fresh frozen brain containing the entorhinal region were obtained and compared. Quantitative autoradiography of 5HT1A receptors was performed using [3H]8-OH-DPAT. Non-specific binding was determined by incubating consecutive sections in the presence of 10μM 8-OH-DPAT. Preliminary analysis of the autoradiograms using computerized image analysis did not reveal any statistically significant differences between patients with schizophrenia and other groups, confirming a prior study of schizophrenics and suicides of this brain region.

**477.3**


The relationship between the expression of 5-HT1A receptors and the level of receptor mRNA was examined in coronal sections of brain from Sprague-Dawley rats. Sections were labelled with [125I]-8-OH-PHAT to visualize 5-HT1A receptors. Adjacent sections were labelled with a 183 base 5S labelled ribogene complementary to rat 5-HT1A. Preliminary experiments demonstrated that [125I]-8-OH-PHAT selectively labels 5-HT1A receptors. In the hippocampus and cortex, the density of receptors decreased significantly between days 3 and 21. After day 21, receptor density increased in CA1, plateaued in CA3 and decreased in cortex. The pattern of mRNA expression paralleled the expression of receptors in each region between days 3 and 21. However, mRNA and receptor expression diverged in all of these regions between days 21 and 60. In contrast, the density of receptors in the thalamus increased between days 3 and 14 and then declined. The density of receptors in the cerebellar cortex declined steadily to almost undetectable levels between days 3 and 60. The pattern of mRNA expression paralleled receptor expression in both of these regions at every age. These results suggest that 5-HT1A receptor expression is directly related to mRNA levels during development. However, it is likely that additional mechanisms regulate receptor expression in the mature brain. (Supported by USPHS MH 43821 and MH-48125)

**477.4**


Corticosterone (CORT) appears to have a role in regulating the 5-HT system. Stress, resulting in increased circulating CORT, acutely facilitates 5-HT turnover. Studies in adult rats have shown that following acute adrenalectomy (ADX) 5-HT1A receptor density is elevated. Cort replacement at the time of ADX maintains the normal levels of receptor density and hippocampal 5-HT1A receptor mRNA expression. In the neonatal rat between post-natal day (pnd) 4-14 CORT levels are normally low and non-circadian. The adrenal is hypersensitive to endogenous and exogenous ACTH. This period is designated as the stress-hyporesponsive period (SHRP). In these experiments we studied the role of CORT on the 5-HT1A receptor during development in order to determine the role of CORT at the 5-HT system. The 5-HT1A receptor density in the rat hippocampus and brain stem was examined at pnd 6 (in the SHRP) and pnd 21 by in vitro binding studies using the selective 5-HT1A agonist [3H]8-OH-DPAT. These studies showed that administration of corticosterone (CORT) reduced 5-HT1A receptor occupancy in the hippocampus and brain stem. In contrast, the 5-HT1A receptor density in the hippocampus and brain stem was examined at pnd 6 (in the SHRP) and pnd 21 by in vitro binding studies using the selective 5-HT1A agonist [3H]8-OH-DPAT. These studies showed that administration of corticosterone (CORT) reduced 5-HT1A receptor occupancy in the hippocampus and brain stem. In contrast, the 5-HT1A receptor density in the hippocampus and brain stem was examined at pnd 6 (in the SHRP) and pnd 21 by in vitro binding studies using the selective 5-HT1A agonist [3H]8-OH-DPAT. These studies showed that administration of corticosterone (CORT) reduced 5-HT1A receptor occupancy in the hippocampus and brain stem.
547.7

CHRONIC ESTROGEN TREATMENT OF FEMALE RATS ENHANCES 5-HT1A-MEDIATED ADAPTED AMPLIFICATION IN HIPPOCAMPAL SLICES. Y. Chen*, K.A. Berg and W. P. Clarke. Departments of Pharmacology and Anesthesiology, Mount Sinai School of Medicine, CUNY, New York, NY 10029.

Female sex hormones and 5-HT1A receptor systems have been implicated in the pathophysiology of affective disorders such as anxiety and depression. Recently, we have shown that chronic treatment of ovariectomized (OVX) female rats with estrogens (E) enhances 5-HT1A-mediated electrophysiological responses in rat hippocampal slices. E-treatment also enhances the 5-HT1A receptor binding of adenylate cyclase in hippocampal membranes, but to a much smaller degree. To study effects of E on both responses coupled to the 5-HT1A receptor in hippocampus we have developed a methodology to assess adenylyl cyclase activity in rat hippocampal slices that are maintained under similar conditions as for electrophysiological recordings. O VX female rats received sc implants of either cholesterol (controls) or 10% 17b-estradiol in cholesterol. Treatment of females with 5-days E (1 mg/kg i.p.) resulted in a significant increase in 5-HT1A receptor-binding of 2-3 fold. In contrast, 5-HT1A-mediated inhibition of forskolin-stimulated AMP-activated (F5CA) was not altered after 1 day E treatment in vivo nor after 30 min or 2 hr treatment with 10 nm E in vitro. In preliminary experiments we have begun to test the hypothesis that the effect of E on 5-HT1A-mediated signal transduction may be mediated by inhibition of protein kinase C (PKC), which can desensitize the 5-HT1A receptor system. 5-day E treatment increased maximal PKC activity measured in vitro in membrane and cytosol fractions of hippocampus. Western blot analysis revealed small increases in the quantity of PKC in hippocampal cytosol and membrane fractions and a slight decrease in PKC in hippocampal membranes in response to E treatment. Treatment of hippocampal slices from OVX rats with the PKC inhibitor staurosporine for 90 min did not mimic E's effect on 5-HT1A mediated signal transduction. More work is needed to evaluate a role for PKC as a mediator of the E effect on 5-HT1A-mediated signal transduction. (Supported by HD46347 and MH48415).

547.8

CHRONIC TREATMENT WITH 5-HT1A RECEPTOR AGONISTS INCREASES 5-HT1A RECEPTOR BINDING IN HIPPOCAMPAL AND CORPUS CALLOSUM SLICES. J. Adrian, J. Rosay and C. Mangenot. Departments of Pharmacology and Psychiatry, University of Montreal, Montreal, QC H3G 1A4.

To determine the effects of chronic treatment with 5-HT1A receptor agonists on binding of 5-HT1A receptors, homogenates of rat hippocampal and corpus callosum slices were incubated in the presence of [3H]-5-HT1A receptor antagonist and increasing concentrations of unlabelled 5-HT1A agonists. The following agonists were used: 8-OH-DPAT, mCPP, buspirone, clozapine, G177, 8-cyclopentyl-1,3-dipropyladenine, a 5-HT2 receptor agonist. The results showed that only 8-OH-DPAT increased 5-HT1A receptor binding in both hippocampal and corpus callosum slices. At 10 nM, 8-OH-DPAT increased 5-HT1A receptor binding by 20% in hippocampus and by 30% in corpus callosum. In hippocampus, the highest increase was observed with 100 nM 8-OH-DPAT. The parallel increase in the binding of 5-HT1A receptor antagonist, [3H]-5-HT1A receptor agonist and 8-OH-DPAT suggests that the increase in 5-HT1A receptor binding is due to a specific increase in the number of 5-HT1A receptors. The specificity of the increase in 5-HT1A receptor binding was further supported by the lack of effect of other 5-HT2 receptor agonists. These results suggest that chronic treatment with 5-HT1A receptor agonists may increase the number of 5-HT1A receptors in hippocampus and corpus callosum and may contribute to the pathogenesis of schizophrenia.

GRECC, SVAMC, and the Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98108.

The 5-HT1A and 5-HT1B receptors were initially described as presynaptic autoreceptors which regulate the release of serotonin. However, these receptors have also been found to regulate the release of several other neurotransmitters in various regions of the rat brain. In this study we investigated the effects of chemical lesioning of serotonergic systems on the levels of mRNA expression in the presynaptic 5-HT1A and 5-HT2B receptors.

5-HT1A mRNA expression (5,7-DHT) or vehicle was infused sequentially into the fourth and lateral ventricles using standard protocols. Serotonergic neurons were identified using 5,7-DHT autoradiography. The mRNA expression levels did not appear to be altered by the 5,7-DHT lesions. In the stratum and CA1 regions of the hippocampus, 5-HT1A mRNA levels did not appear to be altered. However, there was a greater than two-fold increase in hybridization signal density detected for both receptor mRNAs in the dorsal raphe, a major source of serotonergic projections to the forebrain. The increase in mRNA levels detected in the dorsal raphe may reflect spreading of new axonal processes and increased synthesis of axonal proteins.

These results suggest that these receptors are differentially regulated when expressed in presynaptic vs. postsynaptic neurons, and that the postsynaptic receptor mRNA levels can be relatively insensitive to decreases in endogenous ligand concentration.

5-HT1A AND 5-HT1B SEROTONERGIC RECEPTORS MEDIATE HYPERTHERMIA FOLLOWING LONG-TERM TREATMENT WITH 5-HT2A AND 5-HT2C RECEPTOR ANTAGONISTS IN RATS: Pascal Marcel-Pomietto, Chachiquen S. Aakahl, James Tollever*, and Dennis J. Murphy. Lab of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

We have recently demonstrated that hyperthermia induced by (1) 1-2((5-dimethylamino-4-iodophenyl)-2-aminopropane (DOI) and (2) 5,7-dihydroxytryptamine (5,7-DHT) or vehicle was infused sequentially into the fourth and lateral ventricles using standard protocols. Serotonergic neurons were identified using 5,7-DHT autoradiography. The mRNA expression levels did not appear to be altered by the 5,7-DHT lesions. In the stratum and CA1 regions of the hippocampus, 5-HT1A mRNA levels did not appear to be altered. However, there was a greater than two-fold increase in hybridization signal density detected for both receptor mRNAs in the dorsal raphe, a major source of serotonergic projections to the forebrain. The increase in mRNA levels detected in the dorsal raphe may reflect spreading of new axonal processes and increased synthesis of axonal proteins.

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5-HT1A and 5-HT1B receptors are unique among autoreceptors coupled to guanine nucleotide-binding proteins in that chronic treatment in vivo with agonists as well as antagonists decreases receptor density. The decrease in receptor density is thought to be related to down-regulation of the serotonin receptor, the ability of agonists and antagonists to alter receptor density was examined in three heterologous expression systems transfected NIH 3T3 cells with a cDNA for the transfected Madin-Darby Canine Kidney and transfected AT-20 cells. All three transfected cell lines exhibited pharmacological properties consistent with that predicted for cells expressing the serotonin receptor. However, the three cDNAs cloned from different cell lines and transfected into NIH 3T3 cells had different receptor regulation properties after treatment with drugs acting at the serotonin receptor. In transfected NIH 3T3 cells, neither agonist nor antagonist treatment altered receptor density. Treatment with agonist as well as antagonist led to up-regulation of the serotonin receptor in transfected Madin-Darby Canine Kidney cells. In transfected AT-20 cells, treatment with agonist led to receptor down-regulation, whereas antagonist treatment increased receptor density. Thus, the cellular background in which the serotonin receptor is expressed appears to determine the regulation properties of the receptor.


477.19
MOLECULAR MECHANISM OF AGONIST-INDUCED REGULATION OF THE RAT 5-HT2 RECEPTOR. A.C. Chen,* B.D. Ciocanelo, Nancy Ptzinker Lab, Dept. of Psychiatry, Stanford University, Stanford, CA 94305.

The rat serotonin 2 (5-HT2) receptor is expressed in a wide variety of neuronal and non-neuronal tissues. Interestingly, the regulation of the 5-HT2 receptor in response to agonist exposure differs in the various cell types. In primary cultures of rat uterine myocytes, the 5-HT2 receptor is involved in the regulation of collagenase expression; exposure of the cultures to 5-HT2 agonists results in the up-regulation of both the 5-HT2 receptor and collagenase. We present data demonstrating that the agonist-induced regulation of the 5-HT2 receptor is mediated by protein kinase C (PKC) and results in an increase in 5-HT2 mRNA. Furthermore, this up-regulation by agonists (and PMA) is blocked in the presence of PKC inhibitors such as staurosporine and chelerythrine. Experiments addressing whether this regulation is at the transcriptional or post-transcriptional level are currently underway and the results will be presented. In addition, we have recently described the existence of a gene coding for a possible receptor subunit. The cloning and characterization of promoter elements required for expression of the receptor gene in different cell types. Promoter deletion plasmids will be utilized to define the cis-sequences required for affecting the agonist-induced increases in 5-HT2 mRNA levels.

477.21
DOWN-REGULATED CORTICAL 5HT2KETANSERIN BINDING IN ANOREXIC MUTANT MICE EXPRESSING SEROTONERGIC HYPERINNervation. E.S. Camp*, N.M. Tim, T.H. Lab and L.H. Sop, Burke Medical Research Institute and Bourne Laboratory, Cornell University Medical College, White Plains, NY 10605, and Neuropsychology Program, Queens College, CUNY, Flushing, NY 11367.

The murine recessive anorexia mutation anorexia (anore) is characterized by pre-weaning weight loss resulting in death at about 21 days. Weight differences can be seen as early as post-natal day (PD) 6, and by PD 16 mutants (anore) can weigh as little as 30% of normal littersmates. Anorex mice display dysfunctions in ingestive and motor behaviors that are consistent with defects in the central serotonergic systems. Previously, it was demonstrated that the anore mutation is associated with profound serotonergic hyperinnervation in frontal cortex and olfactory areas. We now report that in 20-day-old anorex mice, specific 5HT2Ketanserin binding in the frontal cortex is reduced in comparison with normal littersmates. In vitro autoradiographic binding methods and computerized image analysis were employed. Binding parameters were determined using nonlinear regression assuming a single-site model. Differences were assessed using t-tests (mice per group, *p < 0.02).

Kd (nM) Bmax (fmol/mg)
Normal 12 ± 3 145 ± 15.0
Mutant 7 ± 1.5 71 ± 9.3 *

Although Kds were slightly higher than would be predicted, the location of 5HT2Ketanserin binding sites in the frontal cortex strongly suggests that binding is to 5HT2 receptors. There were 51% fewer receptors in the frontal cortex of the mutant mice compared to their normal littermates. Since serotonin hyperinnervation is a key feature of the anore mutation, the reduced receptor number most likely represents down-regulation of post-synaptic 5HT2 receptors in response to high synaptic levels of serotonin.

Supported by DK26687

477.20
DIFFERENTIAL REGULATION OF SEROTONIN 5-HT1A AND 5-HT1B RECEPTORS AFTER CHRONIC TREATMENT WITH CLOZAPINE, CHLORPROMAZINE AND SOME NOVEL PUTATIVE ATYPIcal ANTIPSYCHOTIC DRUGS. M. Kuppaamaki, E. P. Päivimäki, E. Syvälahti and J. Hieta*, Dept. of Pharmacology, University of Turku, FIN-20520 Turku, Finland.

Previous studies have suggested that 5-HT1A and 5-HT1B receptors may be important for the atypical effects of clozapine. We have used quantitative autoradiography to study the number of 5-HT1A and 5-HT1B receptor binding sites after chronic treatment (14 days, s.c. injections once a day) with saline (SAL, 1 ml/kg), clozapine (CLOZ, 25 mg/kg), chlorpromazine (CPZ, 15 mg/kg), ORG 5222 (ORG, 0.1 mg/kg), risperidone (RIS, 0.3 mg/kg), and amperozide (AMP, 5 mg/kg). Receptor binding was measured 68h after the last injections.

In the doses used, CLOZ, CPZ, and ORG decreased the frontal cortical 5-HT1A receptor binding of 5HT1Ketanserin and 1H[3H]DOB by 40-60%, AMP also significantly decreased 5-HT1B receptor 5HT1Ketanserin binding by 30%, whereas RIS did not affect 5-HT1B receptor binding. In contrast to 5-HT1A receptors, only CLOZ decreased significantly (by about 50%) the 5-HT1B receptor 5HT1Ketanserin and 1H[3H]DOB binding in the choroid plexus. For comparison, we also determined the number of striatal D2 and D3 receptor binding sites with [3H]spiperone and [3H]spiroperone, respectively. CPZ was the only drug to significantly upregulate D2 receptor binding sites. None of the drugs affected D3 receptor binding to any extent. In conclusion, this study suggests that chronic treatment with CLOZ, CPZ, ORG, RIS and AMP differentially regulate 5-HT1A and 5-HT1B receptors.

477.22
REGULATION OF THE 5-HT6 SEROTONIN RECEPTOR IN STABLY TRANSFECTED 293 CELLS. Steven J. Marx*, Frederick J. Montminy, Jr. and David R. Smith, Molecular Neuropharmacology Section, Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892 and Hoffman-LaRoche, AG, Basel, Switzerland.

We investigated the regulatory properties of the cloned rat 5-HT6 serotonin receptor in stably transfected HEK-293 cells. The cell line was characterized using the radioligand [3H]-lysergic acid diethylamide ([3H]-LSD) and exhibited Kd and Bmax values of approximately 5 nM and 1 pmol/mg protein, respectively. Exposure to 5hydroxytryptamine (5-HT) resulted in a dose-dependent stimulation of adenyl cyclase activity in both intact cell and membrane preparations. Stimulation of cAMP accumulation by 5-HT was maximal at 100 μM with an EC50 of 1.5 μM. Pretreatment of the cells with 100 μM 5-HT for 20 hours resulted in a sixty percent decrease in the maximal response for cAMP accumulation with no appreciable change in the EC50. This effect was time-dependent, reaching maximal levels at 8 hours with a half-life of 1 hour. The 5-HT pretreatment appeared to have no effect on the maximum binding capacity of 5-HT-LSD nor the Kd value. Treatment of the cells with various intracellular activators of protein kinases had mixed effects on 5-HT6 receptor function. Exposure to 1 μM phorbol-12-myristate-13-acetate (PMA), an activator of protein kinase C, for 20 hours appeared to have no effect on receptor function or binding capacity. On the other hand, treatment with 1 mM 8-(4-chlorophenylthio)-cAMP (8-CPT-cAMP), an activator of cAMP-dependent kinase protein, resulted in a 55% reduction in the maximal 5-HT cAMP response with no apparent change in potency. In contrast, there was no effect on the Bmax nor the Kd as measured by [3H]-LSD binding. Overall, the data suggest that agonist-induced desensitization of the 5-HT6 serotonin receptor occurs via stimulation of cAMP-dependent protein kinase as measured by a decrease in maximal cAMP accumulation. Furthermore, there does not appear to be a concomitant agonist-induced down-regulation of the 5-HT6 receptor as detected with [3H]-LSD.
**478.1** DISTRIBUTION OF TRYPOTSYLINE HYDROXYLASE IMMUNOREACTIVE AXONS AND CELLS IN RAT MELANOTROPHES AND GLIAL-LIKE CELLS OF THE RAT PITUITARY INTERMEDIATE LOBE. B.M. Chromwell\(^{1}\) and K.A. \(^{2}\) Stricker. \(^{1}\) School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO; \(^{2}\) Department of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

The majority cell population in rat pituitary intermediate lobe (IL) is the melanotrope, a chromophobic-endothelin- and e-NOS-positive cell. Melanotrope secretion is regulated by tonic inhibition via dopaminergic axon terminals. A smaller population of glial-like cells is present in the IL, and synaptic contacts have been described between these IL axon boutons in amphibian and rat. This study utilizes image analysis methods to evaluate the anatomical relationship of tyrosine hydroxylase (TH) immunoreactive axons to cellular elements in the rat IL. Processes were fixed and VIBATOLN stained at 75 µm. Sections were incubated in anti-TH serum, processed for immunohistochemistry, and embedded in Epon. One micron serial sections were toluidine blue stained. Individual melanotropes associated with TH immunoreactive axons were identified, digitally imaged, and followed through successive sections. This process was repeated with individual glial-like cells associated with immunopositive axons. Melanotropes among other melanotropes exhibited lower numbers of TH immunoreactive boutons than those sharing cell membrane surface with glial-like cells. Morphometric measurement of all membrane surface area in single sections revealed higher densities of immunopositive axons associated with glial-like cell structural than with individual melanotropes. Using Image-1 image analysis software, three-dimensional reconstruction of individual melanotropes and glial-like cells indicate that TH immunoreactive axons were closely apposed with glial-like cell processes and followed these processes over several microns. These observations suggest interactions between dopaminergic axons and glial-like cells of the rat IL.

**478.2** GLUTAMIC ACID DECARBOXYLASE: mRNA IS EXPRESSED IN BOTH EXCITATORY AND INHIBITORY NEURONS IN CULTURED HIPPOCAMPAL NEURONS. M.A. Dichter\(^{3}\), Y. Cao, K.S. Williams, \(^{4}\) J. Eberberg. \(^{3}\) Dept of Neurology, Pharmacology, and Psychiatry, Univ. of Penn. School of Medicine and the Graduate School of Medicine, Philadelphia, PA 19104.

The recently developed procedure of single cell mRNA amplification, when coupled with the whole cell patch clamp technique, can be used to investigate the expression and distribution of mRNAs of a variety of proteins in identified cell types. The goal of the present set of experiments was to develop specific molecular "profiles" of excitatory and inhibitory neurons. It was hypothesized that the mRNA encoding glutamic acid decarboxylase (GAD), the enzyme which catalyzes the conversion of glutamic acid to GABA, would be present in detectable quantities only in neurons which utilize GABA as a neurotransmitter. Experiments were performed in low density hippocampal cultures. The whole cell patch clamp technique was used to record from monosynaptically connected pairs of neurons. The presynaptic neurons were positively identified as either excitatory or inhibitory based on the reversal potential and waveform of the evoked postsynaptic current. Excitatory neurons release neurotransmitter which activates postsynaptic glutamate receptors, whereas inhibitory neurons use GABA as a neurotransmitter. Ten neurons were identified as inhibitory and six neurons were identified as excitatory. Initials of the neuronal types were posttranscriptionally regulated. Supported by AG8990 (JE).

**478.3** COMPARISON OF GLUTAMIC ACID DECARBOXYLASE (GAD) IMMUNOREACTIVITY TO DORSOLATERAL STRIATAL (DL-DN) NEURONAL RESPONSE AND UNRESPONSIVE TO CITOCINIC AGONISTS E.M. Sveriges*\(^{3}\), W.C. McDannel and J.P. Gallaghier. \(^{1}\) Dept. of Pharm. & Toxic., \(^{2}\) Univ. of Texas Med. Prep. School, Houston, TX 77030. 

Using intracellular recording, we have previously reported that nicotine and 1,1'-methyl-4-phenylpyridazine (DMPP) hyperpolarize the majority of DLN neurons by a postsynaptic mechanism (Sveriges et al., 1986). We have now compared the morphology, electrophysiology, and GAD immunoreactivity of DMPP responsive (RES) and unresponsive (UNR) neurons.

Neuron (1,2,3), injected was injected into neurons at the end of recording sessions. After cutting the fixed tissue into 75 µm sections, neurons were visualized using the ABC method (Vector) with DAB as the chromogen. Sections containing stained neurons were processed for the GAD immunohistochemistry using a primary antibody at 1:500 (Hoshino et al., 1989) or 1:2000 (Chemicon) dilution. Vector SG was the secondary chromogen. Of the 36 neurons visualized, 18 were DMPP RES (61%). Neither membrane potential nor input resistance were significantly different between DMPP RES and UNR neurons. Furthermore, no other electrophysiological differences were observed, such as the occurrence of low threshold calcium action potentials. AHPs following single action potentials or slow ADPs following a train of action potentials. DMPP RES cells were either multi- or bipolar whereas the DMPP UNR neurons were all multipolar. While no morphological differences were apparent between multipolar DMPP RES and UNR cells, all of the DMPP UNR cells tested were GAD-negative whereas only 60% (6/10) of the DMPP RES cells were GAD-negative. In summary, DMPP UNR cells appear to be GAD-negative, multipolar neurons. Supported by DHHS 1F31 DA00472 to EMS and The Council for Tobacco Research, USA, Inc. to JPG.


The distribution of MAO-A gene products in the adult rat brain and adrenomedulla (AM) was examined by in situ hybridization and by immunocytochemistry with a polyclonal antiserum. The highest levels of MAO-A mRNA and protein expression were found in the locus ceruleus; moderate levels were detected in the serotonergic dorsal raphe nucleus (DRN), habenula, and the adrenergic neurons of the medulla oblongata; low levels were found in the substantia nigra compacta (SNc), hypothalamus, hippocampus, and the cortex. Northern blot analysis of DRN and regions confirmed this distribution. Compared to young adult rats, aged rats showed a clear increase in MAO-A mRNA in the SNc, DRN, and AM by in situ hybridization. In vitro experiments using the rat PCT cell line revealed no significant upregulation of the MAO-A gene in response to the protein kinase (A) activator, forskolin. This is in contrast to the rapid increase observed in tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) mRNAs. PAF-deficient phenocytomatic cell lines showed normal MAO-A mRNA levels but decreased TH and DBH levels, suggesting that the basal transcription of the MAO-A gene is not dependent on cyclic AMP. Promoter analysis of the MAO-A gene will further delineate the response to interacting elements in this important gene.

Supported by NIH grants 48866, 39085, 37020, 00796 and the Welin Professorship to J.C.S.

**478.5** CLONING OF A NOVEL MONOAMINE OXIDASE (MAO) cDNA FROM TROUT LIVER. K. Chen, H.-F. Wu, G. Grishbny and J.C. Shih. \(^{1}\) Dept. Mol. Pharmacol. and Toxicol., \(^{2}\) Sch. of Pharmacy, Univ. of Southern California, \(^{3}\) 1985 Zonal Avenue, Los Angeles, CA 90033.

A trout liver monoamine oxidase (MAO) cDNA was cloned by screening a cDNA library with a human MAO cDNA probe. The trout MAO cDNA encodes 499 amino acids with a molecular weight of 56.6 kDa. The deduced amino acid sequence between trout MAO and human MAO showed 79% and 71% identity respectively. Trout MAO contains the pentapeptide sequence Ser-Gly-Gly-Cys-Tyr motif in which the cofactor FAD is covalently bound. Transient expression in COS cells expressing all three mammalian MAO oxidizes both serotonin (5-HT) and 4-phenylethylamine (PEA), unlike human MAO or MAOB which oxidizes only 5-HT or PEA, respectively. The Km for 5-HT is 9.3 µM for trout MAO (180.7 µM and human MAO (119.3 µM). The Km for PEA is similar for trout MAO (4.8 µM) and human MAO (4.6 µM). When SHET was used as a substrate, similar to human MAO, the Km for this substrate was 2.7 µM and 6.6 µM for human MAO (2.7 µM and 6.6 µM). When using PEA instead of TS, results indicated that trout MAO displays substrate and inhibitor selectivities that are identical to those of human MAO and therefore represents a novel type of MAO. The structure of trout MAO will provide insights into the substrate and inhibitor selectivity of the MAOs. (Supported by NIMH grants R33 MH59983 (MERIT Award), R01 MH37020 (Research Scientist Award), K05 MH00796 and Welin Professorship).

**478.6** DYNAMICS OF SEROTONIN-DEGRADATIVE PATHWAYS IN THE BRAIN OF SOME FROGS. Naokuni Takeda*, Department of Biotechnology, COSMOS Research Institute, Satte, Saitama, 340-01, Japan.

Butofenate (BUTN) has been found in the brain of Bufo bufo japonicus (Soc. Neurosci.19,1171; Comp.Biochem.Physiol.107C,275,94). The main pathways are as follows: Serotonin - N-methyl serotonin (N-MET) - BUTN and serotonin (N-MET) - di-methyltryptamine (DM). In the brain of Rana japonica, serotonin was degraded to N-MET, but not to BUTN. The pathway to DMT was not found. By the injection of NILAMIDE into the body cavity, amounts of each monoamine were highly increased. Furthermore, N-MET was gradually degraded to BUTN, and the pathways to DMT were also detected. In Bufo, NILAMIDE was directly injected into the brain by microdialysis. The amounts of N-MET, BUTN and DMT were highly increased. The monoamine oxidase inhibitor was shown to evoke the appearance of intrinsic metabolic pathways from Serotonin to BUTN and to DMT.
478.7
LOCALIZATION OF TRANSMITTER RECEPTORS IN THE CHICK BRAIN IN RELATION TO AUDITORY IMPRINTING. B. Schnebel and K. Braun. *Federal Institute for Neurobiology, 39118 Magdeburg, FRG.*

The learning process of auditory imprinting in the domestic chick is associated with a significant, regionally restricted increase in the incorporation of radioactive deoxyglucose in the rostro-medial neostriatum/hypothalamus (MNDA), the lateral septal division (LSP) and the ventral tegmental area (VTA) of 17-day-old embryos. The MNDA is the site of initial imprinting and of the VTA the site of memory consolidation. In situ hybridization experiments revealed that the mRNA of the GABA receptor subunits A, B1, and B2 is present in the same brain regions. The results indicate that the GABAergic system plays a role in the learning process of auditory imprinting.

478.8
NMDA RECEPTOR IMMUNOSTAINING IN MOUSE DORSAL COCHLEAR NUCLEUS AND CEREBELLUM. M. Bilak, S. Bilak, and K. Mostert*. Anatomy Dept., Univ. CT Health Ctr., Farmington 06030.

With mAb to NMDAR1 (Pharmingen) light microscopic labeling (Vestacast) of cell surfaces was the same in 3 ICR adults. In cerebellum Purkinje cell body staining varied from intense to negative; dendritic staining was only on 1/2 shafts. No labeling of 3' dendritic, synaptic, or glial structures was evident in the molecular layer. Golgi II cells were stained with negative results. In the intense labeling was associated with granule cell bodies, dendrites, and rosettes. In dorsal cochlear nucleus staining of fusiiform cell bodies varied; their dendritic shafts were negative. In the molecular layer there was an intense pattern resembling that of the 3' dendrites reflecting the synaptic form of parallel fibers, as seen by synaptophycin immunostaining. Granule cells were negative. There was also staining of cartwheel, core, and giant cell bodies and dendrites. We conclude that a significant component of the NMDA receptors differs in the granule cells, inhibitory interneurons, and projection neurons of cerebellum. Also, in the cochlear nucleus, there may differ reflecting roles for NMDAR1 in the processing carried out in these different circuits. Supported by NIH grants R01DC12716, 132DC00025.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
COMPARATIVE IMMUNOCHEMISTRY OF GLYT1 AND GLYT2 GLYCINE TRANSPORTERS IN RAT BRAIN.

1. Introduction

GLYT1 and GLYT2 are two glycinergic neurotransmitter transporters that are localized in the central nervous system (CNS). They are involved in the regulation of neuronal excitability, and their expression patterns are substantially different across different brain regions. Understanding the functional significance of these differences is crucial for elucidating the role of glycine in various neurological disorders.

2. Methods

We used immunohistochemical techniques to measure the expression of GLYT1 and GLYT2 in various brain regions. The antibodies used were raised against recombinant protein sequences specific to each transporter. The brains were harvested and cryoprotected, and then sectioned on a cryostat. Immunohistochemical staining was performed using standard protocols, and the images were acquired and analyzed using confocal microscopy.

3. Results

GLYT1 and GLYT2 are expressed throughout the CNS, with notable differences observed between different brain regions. GLYT1 is predominantly expressed in the dorsal horn of the spinal cord, while GLYT2 is more abundant in the cerebral cortex. Furthermore, GLYT2 expression is found in higher densities in specific areas such as the hippocampus and the cerebellum, possibly indicating a role in synaptic plasticity and learning.

4. Discussion

The differential expression patterns of GLYT1 and GLYT2 suggest a functional specialization within the CNS. GLYT1 might play a role in the tonic inhibition of spinal dorsal horn neurons, whereas GLYT2 could be involved in the modulation of neuronal activity in the cerebral cortex. These findings open new avenues for understanding the role of glycine in neurological disorders, such as pain and addiction.
478.19

AUTORADIOGRAPHIC DISTRIBUTION OF NICOTINIC RECEPTOR SITES Labeled with [3H]Pyridine in the HUMAN BRAIN: COMPARISON WITH MUSCARinic RECEPTORS. L. LeGates, D. Chen, C. Przyborski, and R. Lescot. Department of Neuroradiology, School of Medicine, University of Massachusetts Medical School, Worcester, MA.

The localization of central nAChRs and nicotinic receptor sites might provide insights into the organization of the cholinergic system in various brain areas. Using in vitro autoradiography, the distribution of nAChR ([3H]Pyridine, 20 nM; [3H]Hepazine, 15 nM) and M3 ([3H]AF-DX 384, 2 nM) binding sites was investigated in postmortem human brains from the frontal, temporal and occipital cortices, the basal ganglia, the thalamus, and the cerebellum.

478.20

A Volskenin lesion of striotonal neurons produces a marked decrease in mRNA expression for the m4 subtype of the muscarinic acetylcholine receptor. *M. Tissot et B.H. Ulley, UVA, Charlottesville, VA 22908 and DVMAC and Vanderbilt Univ., Nashville, TN 37212

By in situ hybridization, 3 subtypes of the muscarinic acetylcholine receptor are expressed in rat striatum, the m1 subtype in the majority of striatal neurons and the m2 subtype, a probable autoreceptor, in cholinergic interneurons which also express the m3 subtype. The m3 subtype is consistently expressed on striatal neurons which express PPE mRNA but only in a subgroup of neurons expressing PPE mRNA.

479.1


By whole cell patch clamp, somatostatin (SRIF) (0.1 μM) significantly decreased glutamate sensitivity to a pertussis toxin-dependent mechanism in neuronal cultures from foetal (E16) mouse hypothalamus after the onset of neuronal maturation (14 days in vitro). At this time, five subtypes of G-protein linked SRIF receptor have been cloned in rodent hypothalamic membranes. We recently reported that SRIF receptor are expressed in the developing hypothalamus. At 14 days in vitro, SST3 mRNA levels were twice higher than those of SST2 and SST4 while SST3 and SST5 mRNAs were not detected. By 125I-SRIF receptor radioautography, a small population of hypothalamic neurons (~10%) was endowed with binding sites that are localised on both cell bodies and axonal processes in the hypothalamus. 

479.3


This study examines the recovery of melatonin receptor expression in visual areas of chick brain following suppression by intracarotid TTX administration. Chick embryos (3 weeks old) were injected with either 6 μg TTX or salinevehicle (C), and were sacrificed at day 7 (time 0) or after 1, 3 and 7 days recovery. Brain sections were labeled with [3H]-iodomelatonin. A high density of binding sites localized in the optic tectum (1 mA). Areas ipsilateral to the TTX-injected eye were used as a control. Reductions in specific [3H]-iodomelatonin binding to TTX (time 0) were observed in the contralateral optic tract, primary optic tectum, n. basal optic root, secondary (n. triangularis) and tertiary (ecostolatum) visual areas (also see ref 3). The eye remained depressed for 2-3 days but was completely recovered following TTX. The extent of recovery correlated with the degree of pupillary reflex suppression. We conclude that expression of ML-1 melatonin receptors in chick brain visual areas is selectively modulated by the visual input. MH-42922.

479.4


We previously reported that a high density of 2-5 ml melatonin receptors in the retinoventricular layers of the chick optic tectum (stratum griseum et fibrosum superficiale, SGFS) and lower levels in the optic nerve (ON). Binding in both regions is decreased by ON transection (Brain Res. 590:252-359, 1992), which suggests transport of melatonin receptors from the ON to the retina to terminals in the SGFS. We have examined the electrophysiological effects of melatonin on retinotectal transmission in a brain slice preparation of the chick tectum. Bath application of 10-20 nM melatonin suppressed tectal field potentials by stimulation of retinal afferents at the level of the ON or stratum opticum. Alterations in the presynaptic components of the field potential isolated in the retinotectal preparation in the ON of a presynaptic site of action for melatonin. We have also found that in vivo intracarotid injections of tetrodotoxin (TTX, 6 μg), which blocks the electrical activity of the retinal ganglion cells, decreases 2-5 ml melatonin binding in the ON and optic tectum to a similar extent as that seen following transection. TTX (in one eye of 3 week-old chicks, every 3 days for 7 days) decreased binding in the contralateral ON (25%), SGFS (20%) and ipsilateral ON (18%) and layers: 58%, inner layers: 38%) and stratum griseum centrale (34%). Binding in these areas recovered to control levels by 3-7 days following cessation of the TTX treatment. This suggests that expression of melatonin receptors in the retinotectal pathway, including presynaptic receptors which modulate visual input, is regulated by the activity in the pathway. (Supported by NS 24560 and EY 06860 to HJG and MH-42922 to MLD.)
479.5
DOPAMINE RECEPTORS ARE ELEVATED IN SCHIZOPHRENIA AND ALZHEIMER'S (AD) CASES

The binding of \({}^{3}H\)-7-OH-DA (PIPA; 0.2 nM, or 0.25 nM) to the ligand that binds selectively to D2 dopamine receptors, was analyzed in sections of human brain from AD cases and control subjects. The binding varied from 4 to 8 fmol/100 mg protein in different striatal areas, which is approximately 5-7 times lower than that of D2 receptors. The highest concentration of D2 receptors was observed in the caudate nucleus, the ventral striatum, the pallidus, and the internal part of the globus pallidus (GPi) with slightly lower binding in the external globus pallidus (GPe) and ventral pallidum (VP). The nucleus basalis of Meynert exhibited relatively high binding of D2 receptors. In the striatal region the binding of D2 receptors visualized with \(\text{[}^{3}H\text{]epidepride in the presence of 50 nM of 7-OH-DA. D2 receptor density was significantly decreased in the dorsal caudate nucleus, the ventral striatum, the pallidum, and the GPe regions of the GPi as compared to control.}

The only brain regions outside the striatum that displayed D2 receptor density comparable to that of the striatum were the SN and amygdala. We compared PIBAT binding in caudal striatum in schizophrenia cases (n=12, 76.4±1.2 yrs) with neurologically normal control (n=12; 67.4±3.4 yrs) and AD (n=11; 79±2 yrs). The binding to D2 receptors was significantly elevated in schizophrenics in all striatal areas except for the GPi. The highest increase was observed in the GPi (81%) followed by the nucleus basalis (72% average increase). There were even higher elevations in all regions for AD. The highest increase was observed in the GPi (101%) and ventral putamen (73% average increase) followed by the GPi (63%). The results suggest that the striatal system leads to a selective elevation in D2 dopamine receptors. Funded by MH 43880, AG 09287.

479.6

The effects of anesthetics on neurotransmitters at specific sites in the brain are largely unknown. In this investigation, we studied immunohistochemical expression of 5-HT and FOS in the rat brain. Paired control and experimental male Wistar rats were chronically cannulated for blood pressure monitoring and anesthetic or vehicle infusion 7 days prior to the experiment. Following a 6 hour infusion of propofol (20-25 mg/kg/hr) or the intralipid vehicle, the immunoreactivity for serotonin (5-HT) and FOS were studied. Changes in immunoreactive cells within the raphe regions were quantified using computerized imaging analysis: Fos activity was determined by counting the positively stained nuclei. The 5-HT signal was significantly increased in the dorsal raphe nucleus, and significantly decreased by 15% in the area postrema compared to control. Numerous positively stained foci were observed in the inferior olivae in the experimental animals which was not seen in the controls. The lateral parabrachial nucleus had double the number of labelled nuclei for fos compared to the control. These results suggest that anesthetic such as propofol can evoke specific changes in gene activity and selective changes in neurotransmitter systems which may have functional implications for the autonomic and behavioral effects of anesthetics. (Supported by the Heart and Stroke Foundation of Ontario)

479.7
\(\text{E-ADRENERGIC RECEPTOR AUTORADIOGRAPHIC LABELLING IN HUMAN BRAIN: SUBTYPE DISTRIBUTION, EFFECTS OF AGE AND POST MORTEM DELAY AND MODIFICATIONS IN ALZHEIMER'S DISEASE. N. Vronskaya, S. N. Gudjch**, J. Gonzalez-Ollo, and J. Dussoit. Dept. of Physiology, Fac. of Sciences, Univ. of Cebrià, Sant Cugat, Spain.}

The density and distribution of \(\text{E-}
\text{adrenergic receptor subtypes was quantitatively autoradiography in post mortem human brain, in both control and Alzheimer's disease (AD) cases. The sections were incubated with \(\text{[}^{3}H\text{]tacrine-pindolol (ICP). ICP-8406 and ICP-11855 were used as displaceants. Our results showed a clear predominance of the \(\text{E-}
\text{subtype in areas such as the basal ganglia and the neocortex. E-}
\text{adrenergic receptors were mainly distributed in the cerebellum and hippocampus. There was no effect of post mortem time (4-72h) on the density of ICP binding. In contrast, a general trend of the \(\text{E-}
\text{receptors to decrease with age was observed. However, this decrease was statistically significant in telencephalic areas, such as the basal ganglia, frontal and visual cortex and thalamus, but not in others. A similar study suggested that
\text{the mesencephalon and the cerebellum. This decrease was far noticeable in the cerebellum and hippocampus. The receptor loss was secondary to a decrease of \(\text{E-}
\text{receptors (39-60%). The decrease of \(\text{E-}
\text{adrenergic receptor density during normal aging and in AD can be explained by the neuronal loss besides the degeneration of the locus coeruleus, known to occur in these processes. (Supported by DICYOT SAFP-265).}

479.8
\text{CELLULAR CO-LocalIZATION OF \(\text{a}
\text{AND \(\text{b}
\text{ADRENERGIC RECEPTOR SUBTYPES IN PRIMARY CULTURES OF RAT SPINAL CORD NEURONS. Y. Huang, H.Y. Langston, H.E. Laidt Jr., P. A. St John and P.T. Kepes. Departments of Pharmacology & Toxicology, Physiology, and Anatomy, University of Arizona, Tucson, AZ 85724.}

The results of molecular cloning have revealed three subtypes of the \(\text{a-}
\text{adrenergic receptor. These subtypes have been defined as the \(\text{a}-\text{C10-receptor (a2C10) and \(\text{a}-\text{C4-receptor (a2C4). \(\text{a}-\text{adrenergic receptors have been characterized in the central nervous system and spinal cord where they are involved in a number of physiological activities, including the control of blood pressure and nociception; however, the specific cellular localization and the subtypes which mediate these effects are largely unknown. For each of the \(\text{a}-\text{adrenergic receptor subtypes, polyclonal antibodies were raised in rabbit using recombinant proteins containing the third intracellular loops of these receptors. The antibodies were characterized in transfected COS cells expressing each of the subtypes individually and were found to be selective and did not cross-react. Primary cultures of rat spinal cord were prepared from 14 day embryos. After 9 days in culture, they were examined by immunofluorescent microscopy. Positive immunoreactivity was detected with antibodies to the \(\text{a2A}
\text{and \(\text{a2B}
\text{, not with antibodies to the \(\text{a2C}
\text{). The labeling was on neuronal, but not on glial cells. It was blocked by pretreatment of the antibodies with the appropriate fusion protein. The labeling in both subtypes was distributed diffusely across the cell body and neurites, and about 80% of the neurons in heterogeneous spinal cord cultures showed the labeling in both cases. Using dual-labeling techniques, positive immunofluorescence was co-localized to neurons with both \(\text{a2A}
\text{and \(\text{a2B}
\text{antibodies. The rat spinal cord appears to contain \(\text{a2A}
\text{and \(\text{a2B}
\text{adrenergic receptors which are present on neurons and may be co-expressed in the same cell. (Supported by the ADCRC.)}

479.9
\text{\(\text{a2C10-ADRENERGIC RECEPTOR mRNA IN THE MONKEY CEREBRAL CORTEX: NON-RADIOACTIVE IN SITU HYBRIDIZATION COMBINED WITH IMMUNOCYTOCHEMICAL LABELING OF CELL TYPE-SPECIFIC MARKERS. E. Wenig and M.S. Liao. Section of Neurology, Yale University School of Medicine, New Haven, CT 06510.}

The nonradioactive in situ hybridization combined with immunocytochemical labeling techniques have been used to study the expression of adrenergic receptors in the monkey neocortex. Using a 32P-labeled riboprobe sequence specific for the \(\text{a2C10}
\text{receptor mRNA, the expression was observed in all cortical areas and layers with the exception of the amygdala.}
479.11 Functional Localization of Alpha-2 Receptor function in deminia of the Alzheimer's type and normal aging, regional cerebral blood flow (rCBF) was measured using 15O-H2O PET (Scandinavian PET Center). Five screened healthy controls (aged 20-30, four male, one female, all right-handed) (eyes and ears excluded) and 5 during visual stimulation with isopiloc textures in an alternating fashion. The first two scans (off drug) were followed by a 200 mg/kg 3 minute infusion of iodazoxan, with a maintenance drip for the remaining eight scans. All ten scans for each subject were interpolated from 15 to 43 slices, roll-yaw corrected, regridded, normalized to Talairach space, and smoothed using a gaussian filter of 20 mm x 20 mm x 12 mm. Each scan for each subject was normalized to its own global mean, then multiplied by the mean of the two non drug scans for that subject. Pixel-by-pixel analysis of rCBF was performed for each subject and for the group to identify regions affected by iodazoxan. In addition, changes in rCBF were measured in each subject across time in the regions with the largest drug effect. In spite of a significant decrease in global blood flow across time (P<0.001), blood flow to visual cortex was significantly increased by iodazoxan in the group in both the resting and visually stimulated conditions (P<0.05). Moreover, each subject showed an rCBF increase in visual cortex after iodazoxan in the same areas identified by the group analysis. This metabolic increase is consistent with the high density of alpha-2 receptors in the human visual cortex and enhancement of neurotransmitter release. Intravenous iodazoxan and PET may be a useful way to assay central alpha-2 receptor function in neuropsychiatric illnesses.

479.12 OCTOPAMINE, DOPAMINE AND NORADRENALINE IMMUNOCHEMISTRY IN THE RAT BRAIN: THREE DISTINCT PATTERNS OF DISTRIBUTION. S. Burchett, T.P. Hicks*, J. Rugg, and M. Eckert, Department of Biology and Psychology, UNC, Greensboro, NC, 27412-5001.

The distributions of these three amines normally present in the mammalian nervous system: octopamine (OA), dopamine (DA) and noradrenaline (NA), were compared in rat brain following immunocytochemical treatment with polyclonal antibodies. The distributions of NA and DA conformed well with previously published accounts. The OA-containing cells were confined to the locus coeruleus (A6, A5, A2 and A1 zones) while that for somatic DA was present in the ventral tegmental area and substantia nigra. The development of a novel antibody to perform the first screening study for this compound in mammalian CNS. We found somatic label to be distributed increasingly along the rostro-caudal axis; staining in more caudal regions being relatively intense and widespread than rostrally. OA immunoreactivity was demonstrated best in rats perfused with fixative solutions having relatively high glutaraldehyde levels (6%), causing a high background level of stain, and using low serum dilutions (1:250). Caudally-directed structures showing the most intense reactivity included: cranial nerve nuclei (especially V and VIII); throughout the central core of the long axis of the reticular formation and ventrolateral to the cerebral aqueduct; and the deep cerebellar nuclei. The distribution of label throughout CNS was heterogeneous even though immunoreactive product was dispersed very widely within somatic and all alar/pallidal areas not showing labelled cells. Background label was also quite uneven, suggesting the possibility of unequal patterns of terminal immunoreactivity. These results are not inconsistent with the idea of OA as a modulator of synaptic transmission within mammalian brain.


Nitric oxide (NO) is a highly reactive and diffusible molecule which participates in a wide variety of functions such as the citoexcretory action of leucocytes, as mediator of neurotransmitters in the relaxation of vascular endothelium, and as an inhibitor of platelet aggregation.

From a phylontic point of view, the presence of NO producing neurons has been described in vertebrates such as rat, crab, frog and fish, and in invertebrates like cephaliid and annelids. Thus, the aim of the present study was to report the presence and localization of NO producing neurons in the CNS of the mexican amphibian axolotl, Ambystoma mexicanum. The axolotl (n=6) were anesthetized with benzocaine and intracardially perfused with 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). The brains were dissected and post-fixed for 3-12 h, and were transferred to a solution (15%). Parasagittal and coronal sections (5µm) were stained with NO-stain or NODISHOT (Lichtman, 1989) and double stained with NO-stain or NODISHOT (Lichtman, 1989) and double stained with NO-stain and antibodies.

NO-protein correlation study was performed using different antibodies and different fixatives. Anti-NO-staining and anti-NO antibodies were found in several labeled neurons in the axolotl but no correlation was observed between the two staining methods. In conclusion, NO producing neurons are found in the CNS of A. mexicanum and might play an important role in the functioning of the CNS of this species.

479.14 SEROTONINERIC BUT NOT DOPAMINERGIC FIBERS IN THE STRIATUM OF THE DOPAMINE-RECEPTIVE, POSSIBLE CO-LOCALIZATION OF 5-HT AND CAMP.


Department of Psychiatry and Neuropsychopharmacology, University of Limburg, Maastricht, The Netherlands and Department of Anatomy and Neurobiology, Dalhousie University, Halifax, NS, Canada.

Within the striatum several efferent and afferent transmitter systems can be demonstrated of which serotonergic and dopaminergic varicoses fibers are the most prominent. The major neuronal system in the brain has been visualized using either antibodies to nitric oxide synthase (NOS) or visualization of functional responses. Furthermore, NO- and NADPH-diaphorase positive cells and fibers were localized. However, it is not clear which producing NO fibers are arising from intra- or extrastriatal origin. In this study we were interested which elements are also immunoreactive for the cAMP dependent protein kinase for NO production. We have found that the produced NO will diffuse from the cell body and can activate neighboring neurons guanylate cyclase. This leads to the production of cGMP, and using 8-Br-cAMP-immunohisto化学ly demonstrated that a reduction of the dopaminergic fibers does not lead to a decrease in the number of cGMP-positive fibers, while 5-
DHT lesions induces a diminishing of the serotonergic fibers and cAMP fibers in the striatum. Further studies are in progress to show the possible co-localization between cAMP and cGMP fibers and the absence of co-localization between cGMP and NO fibers in the striatum. This co-localization study provides support for a functional connection between the NO and the 5-HT system (Supported by NWO of The Netherlands).

479.15 NADPH DIAPHRASE ACTIVITY IN OLFACTORY RECEPTOR NEURON AXONS CONFORMS TO A RHINOLOCOPITICALLY-DISTINCT DORSAL PROJECTION ZONE IN THE MOB OF HAMSTERS. T. Kato and T. A. Schonfeld*, Dept. of Psychology and Biology and the Neuroscience Program, Clark University, Worcester, MA, 01610.

NADPH diaphorase histochemical activity is distributed widely in the CNS, where it is most commonly localized to short-projecting neurons whose axons do not collect, for the most part, in the major white matter tracts such as the corpus callosum or corticospinal (Brown and Kimmus, 1991, Neuroscience 46:755). By contrast, the olfactory and vomeronasal nerves show intense NADPH diaphorase activity in the main olfactory bulb (MOB) and accessory olfactory bulb (AOB), respectively. Moreover, the olfactory nerve activity patterns are also topographically restricted to the dorsal and medial MOB (Scott et al., 1987, J. Comp. Neurol. 260:797-809; Crepel and Brunois, 1988, Brain Res. 406:323; Davis, 1991, J. Comp. Neurol. 314:493). This pattern conforms almost precisely to an ethologically oriented, rhinotopically-distant dorsal zone known to receive projections from mucosal segments that line a relatively smooth central channel within the naso cavity (Schoenfeld et al., 1994, Brain Res. Bull., 34:183; Clancy et al., 1994, Brain Res. Bull., 34:211). Thus, NADPH diaphorase activity, positively indicated of nitric oxide activity as well, may play a role in the spatial coding of odorant molecules.

(Supported by an NSF REU site grant, the Colin Research Award and the Department of Psychology, Clark University.)


The presence of NADPH diaphorase activity in neurons is believed to be indicative of the presence of nitric oxide synthase in the CNS. In some avian species (quail, chickens), NADPH diaphorase is present in a series of neurons located in specific areas of the brain. This study is directed to investigating the distribution of this enzyme to the brain of the budgerigar (Melopsittacus undulatus) CNS show any peculiar specific segregation. To this effect, the brains of a series of M. undulatus have been studied for the presence of NADPH diaphorase-containing neurons.

In the telencephalon, the paleostriatal-paleoarchlial lobe complex showed the presence of positive neurons and a diffuse network of axons. Diaphorase-containing elements were observed also in the neostriatum in several areas of the accumbens (including the nucleus accumbens) and in the hyperstriatum (accessory, dorsal and ventral). In the diencephalon, positive neurons were present in the thalamic regions and periventricular areas, and in a segregate area at the circumference of the anterior commissure and the lateral precommissural bundle. A group of positive perikarya was located lateral to the dorsal part of the III ventricle in the paraventricular hypothalamic area. In the mesencephalon, diaphorase-containing elements were placed in the posterior area, reticular formation (pars lateralis and pars medialis), nucleus ruber and nucleus arcuatus. Several positive elements were located in the medullary pedunculi pedalis, nucleus vestibularis (pars medialis and pars superior). In the cerebellum, large stained neurons were evident in the nucleus cerebellaris inferius. NADPH-containing neurons in the budgerigar brain show a specific distribution, only in part overlapping with what already observed in other avian species. In particular, NADPH-containing neurons in Melopsittacus undulatus are largely distributed in several visceral areas except the extroseptum. Differences with what observed in the chicken and quail are evident especially in the hypothalamus and medialbrain.

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479.19
TRANSFERRIN RECEPTOR ANALYSIS IN HYPOTRANSFERRINEMIC (Hp) MOUSE BRAIN. T.D. Dickinson* & J.B. Connor, Penn State Univ., Hershey, PA 17033
The hypotransferrinemic (Hp) mouse has a point mutation or small deletion in the transferrin (Tf) gene causing a defect in splicing of precursor Tf mRNA. This results in production of <1% of the normal circulating level of Tf. These animals are small, pale and severely anemic at birth and die within 7 days unless supplemented i.p. with transferrin. We have previously analyzed the brains of these animals and shown morphological alterations, especially in areas of significant postnatal development. We have also studied the cellular and regional distribution of iron, Tf and ferritin in the Hp brain. An appreciation of the cellular and regional distribution and levels of Tf receptor in these animals is critical to complete our understanding of the mechanisms of iron transport and storage in the Hp mouse brain. Rat iron and monoclonal antibodies to Tf receptor were applied to 45µm brain sections from wild type (Hh), heterozygote (Hp) and mutant (hh) Hp animals. The overall staining for Tf receptor using these antibodies is similar for all three types and staining is generally more robust using the polyclonal antibody. The predominant cell type staining positively for Tf receptor are neurons. The Tf receptor positive neurons are seen throughout the brains of all three types. While matter astrocytes stain Tf receptor-positive but while matter itself reacts poorly. Perivascular astrocytes also stain Tf receptor-positive. Blood vessels themselves, known to contain high level of Tf receptor, stain positive in all three animals, but react more intensely with the monoclonal antibody. Oligodendrocytes, the cells responsible for myelin production in the brain and the predominant iron-positive and Tf-positive cells, do not stain positively for Tf receptor using our antibodies.

480.1
METABOTROPIC GLUTAMATE RECEPTOR-MEDIATED POTENTIATION OF CYCLIC AMP RESPONSES DEPRESSES EXCITATORY SYNAPTIC TRANSMISSION BY A PROTEIN KINASE-INDEPENDENT MECHANISM. R.W. Gereau IV* & P.J. Conn, Depart. of Pharmacology and Program in Neuroscience, Emory Univ., Atlanta, GA 30322
Coactivation of metabotropic glutamate receptors (mGluRs) and β-adrenergic receptors causes a synergistic increase in cAMP formation in the rat hippocampus. Increases in cAMP are known to have many actions in the hippocampus via activation of β-adrenergic-dependent protein kinase. We now report that coactivation of mGluRs with 1S,3S-ACPD and β-adrenergic receptors with isoprenaline induces an acute depression of excitatory post synaptic currents (EPSCs) at the Schaffer collateral-CA1 synapse. Neither 1S,3S-ACPD nor isoprenaline depresses EPSCs in area CA1 of the adult rat hippocampus when added alone. Pharmacological studies indicate that this depression of EPSCs is dependent upon increases in cAMP levels but is independent of protein kinase activity. This CAMP-mediated depression of EPSCs appears to be dependent on metabolism of cAMP and release of adenosine or 5'-AMP into the extracellular space with resultant activation of presynaptic adenosine receptors. These studies suggest that cAMP can have local hormone-like effects in the hippocampal formation that are independent of cAMP-dependent protein kinase.

480.2
METABOTROPIC GLUTAMATE RECEPTOR STIMULATED cAMP IS IMPLICATED IN VISUAL CORTEX PLASTICITY. D.H. Hawkins*, N.W. Davis, D. Gregory, and S. Reid. Dept. of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, CT 06520
Metabotropic glutamate receptor (mGluR) stimulation can activate phosphoinositole (PI) hydrolysis and can increase and decrease cAMP. The mGluD1 linked to PI hydrolysis has been implicated in functional cortical plasticity (Bose and Dedde, Am. Neurosci. Acad. 627: 42-56, 1991). The role of the cAMP mGluRs in visual cortex plasticity has not yet been addressed, although the cAMP second messenger system has been implicated in hippocampal plasticity. This study was undertaken to examine the role of cAMP dependent mGluR's in visual cortex plasticity. The critical period in the rat begins at approximately 14 days of age and extends approximately one month. Consequently, cortical slices (400 µm) were obtained from male Long Evans rats approximately 22 and 50 days of age. After a 90 minute preincubation in a static chamber, slices were transferred and given a 15 minute experimental incubation. Basal cAMP levels were determined from slices incubated in extracellular buffer. Quipiquone (100 µM) or quisqualate (100 µM) were used to stimulate mGluR1 and mGluR5. CAMP was quantitated via RIA. Basal cAMP levels were slightly, but not significantly higher in slices from 22 versus 50 days of age (p < 0.05). Experiments were conducted in the presence of 1 µM mGluR antagonist PD 104059. Under these conditions stimulation by quisqualate increased cAMP levels in slices from 22 days of age in neocortex by 45 fold and in 50 day old rats by 5 fold. This was statistically significant (p < 0.002, unpaired t-test) in the 22 day old rats but not in the 50 day old rats. These results suggest that mGluR1 and mGluR5 stimulation of the cAMP second messenger system is implicated in rat visual cortex plasticity. Supported by EY00053 and EY007115.
DIFFERENTIATION OF PKA IN CORtical NEURONS AND CEREBELLAR GRANULES. C. Venta*, M. Paulillo, A. Porcellini*, A. Feliciello*, V.F. Avvedimento* and G. Schetti*, Dept. of Neuroscience, S. Sect. of Pharmacol., University of Naples "Federico II", Italy. Cortical neurons display a notable amount of type-II holocenzyme compared to cerebellar granules, virtually containing only type-I holocenzyme and devoid of AKAP 150, the type-II PKA holocenzyme. The cortical holocenzyme poorly associates under stimulation (5 min) by forskolin (10µM+IBMX 50µM), while completely reassociates after 15 min; nonetheless, a consistent nuclear accumulation of PKA is found. Conversely, cerebellar holocenzyme is highly sensitive to stimulation by forskolin (>60µM) which does not reassociate at 15 min, and the catalytic subunit does not accumulate into the nucleus. Immunofluorescence studies using a polyclonal antibody for PKA catalytic subunit and band-shift experiments using radio-labelled CRE are allowing us to further unravel this issue. In cortical neurons, the cytosolic holocenzyme is dissociated at 15 min, while the particlect part correspondant dissociates between 2.5 and 5 min and fully reassociates at 15 min; negligible levels of particulate holocenzyme are found in cerebellar granules, and most of cytosolic holocenzyme dissociates under forskolin stimulation (60-70%, 1-15 min). Our results suggest that relevant functional implications could underly the different subcellular distribution and response of PKA to CAMP, with particular regard to the CAMP-dependent modulation of gene transcription, which plays a major role in the modulation of long-term synaptic events. (Supported by TP Aging and CNR 93.00417.CT04 grants to G.S.)

50.5 PROTEIN KINASE A (PKA) INDEPENDENT EFFECTS OF IONTOPHORETICALLY APPLIED CAMP ANALOGS ON RAT SUBSTANTIA NIGRA PARST RETICULATA (SNpr) NEURONS. I.C. Lata*, L.P. Martin and R.L. Waucquier*, Dept. Pharmacological Sciences, Northeastern Univ., Boston, MA 02115. We have previously shown that iontophoresis of the dopamine D1 agonist SKF 38393 acts at D1 receptors on striatal terminals to increase firing rates of SNpr neurons, and to enhance their inhibitory responses to applied GABA. To determine if CAMP might mediate these effects, subsequent studies examined effects of CAMP analogs using extracellular single unit recordings in male rats anesthetized with chloral hydrate. Application of several membrane permeable analogs (B-ionophore-cAMP, dibutyryl-cAMP and chlorophenylthio-cAMP) failed to consistently mimic the rate-increasing effect of the D1 agonist. Moreover, these compounds, as well as the non-permeable CAMP analog Rp-cAMPS, produced significant, but not as large, increases in GABA potency that were not blocked by the cAMP antagonist Sp-cAMPS. These results suggest that CAMP may mediate these effects, by a mechanism that remains to be elucidated. (Supported by NS 23341.)

50.6 CHRONIC HALOPERIDOL TREATMENT HAS NO SIGNIFICANT EFFECT ON CYCLIC AMP CONCENTRATION IN RAT BRAIN. W. Feng, R.H. J. Elkies and R.S. El Mallah*, Mood Disorders Research Program, Department of Psychiatry and Behavioral Sciences, University of Louisville School of Medicine, Louisville KY 40292. It has been assumed that the therapeutic effect of haloperidol in the treatment of schizophrenia is related to its blockade of dopaminergic D2 receptors in the central nervous system. Our previous data has shown that chronic haloperidol treatment attenuates receptor-mediated phosphoinositide turnover in the frontal cortex, striatum and hippocampus. To investigate the influence of haloperidol treatment on another second messenger, cyclic adenosine monophosphate (cAMP), in the central nervous system, we examined the cAMP concentration in the different brain regions of rats treated with haloperidol (1.5mg/kg/day IM) for 4 or 6 weeks. The cAMP concentration was measured by dual range cAMP enzyme immunoassay kits. Our results indicates that cAMP level was slightly increased in the hypothalamus while no alteration was observed in the frontal cortex, striatum and hippocampus after 4 or 6 weeks treatment with haloperidol. However, all these changes are not significant. Chronic haloperidol treatment does not appear to be associated with the changes in cAMP that have been previously reported with acute treatments. Our results provide additional support for the notion that chronic treatment experiments are necessary to understand the mechanism of clinical action of haloperidol.

50.7 SARCOMA PROTO-ONCOGENE c-LYN (P-56) IS HIGHLY EXPRESSED IN THE RAT BASAL FOREBRAIN. S. Chen*, R. Bing and D.E. Hillman, Dept. of Otolaryngol. and Phys./Biophys., New York Univ. Med. Center, New York, N. Y. 10016. The sarcoma proto-oncogenes are second messengers for phosphorylation of tyrosine kinases which are essential in processes of neuronal plasticity and learning. Complete subcellular fractionation mapping and reconstruction of lyn immunoreactivity (IR) revealed impressive bilateral cores of intensely labeled neurons surrounding the anterior commissure and extending along the base of the striatum and the head of nuclei accumbens caudally through the striatal fundus. There was a compartmentation of lyn labeling by nuclei displaying multiple foci consistent with tissue sections. Neurally specific lyn IR was present in this region, intensely labeled small cells were grouped along the margins of the accumbens and the accumbens nucleus. Immunoreactivity in the belt and rudimentary form distinct fascicles. The dendrites of these neurons within the fascicles were oriented parallel forming distinctive dendritic fascicles. These fascicles were also prominent in the shell of accumbens and bed of striatal nuclei. The fundal foci were intensely labeled by a variety of extracellular extension. The lateral and medial septal nuclei were separated by intense IR comuta and overlapping dendritic fields. Additionally, moderate lyn-IR cells having small to medium sized perikarya scattered throughout the neostriatum. Double labeling revealed that a small percentage of lyn cells colocalized with parvalbumin or none colocalized with large acetylcholine cells. The lyn core in the basal forebrain may represent a basic system of neurons involved in learning homeostatic functions of behavior. Supported by NS 13742 and AD 09480.

50.8 MULTIFUNCTIONAL CALCIUM-DEPENDENT PROTEIN KINASE II (CaMKII) IN BOVINE CHROMAFFIN CELLS: CHARACTERISTICS AND STIMULATION BY POTASSIUM DEPOLARIZATION. N. Vera and J.C. Wayne*, Department of Neurobiology and Anatomy, University of Texas, Houston, TX 77030. CaMKII is a multifunctional protein kinase that has been found to exist in a variety of tissues and cell types. We examined CaMKII activity in bovine adrenomedullary chromaffin cells using a CaMKII specific substrate. After discriminating its presence, we investigated the time course of CaMKII activation as determined by incubations at incremental time points with nondepolarized and high potassium depolarized (55 mM KCl) cells. We found that CaMKII activity in chromaffin cells differs both temporally and quantitatively from that in other cell types, such as PC12 cells and hippocampal slices. In chromaffin cells, the activation is almost immediate (occurring within 10-20 min) and rapidly decays by 2 minutes. CaMKII is known to activate tyrosine hydroxylase and phosphatidylating serine residues 19 and 40 in chromaffin cells. The rapid phosphorylation of these residues (maximum phosphorylation within 10 seconds) correlates with the activation of CaMKII. Therefore, in chromafflin cells, the time course of CAMPII activation directly predicts the rapid phosphorylation and activation of tyrosine hydroxylase, and thereby catecholamine synthesis. Supported by NS 11061-16 JCW.
transplantation of calcium/calmodulin-dependent protein kinase II (CaMKII) in rat hippocampal slices is induced by simulated ischemic insult. S. J. Khub and M. W. Watanabe*. Dep't of Neurobiology and Anatomy, Univ. of Texas Med. Sch. Houston, TX 77030. CaMKII has been shown to lose activity and to translocate from post-synaptic to pre-synaptic fractions of cortical and hippocampal homogenates in a global model of ischemia (Aronowski et al., 1992, J. Neurochem.). To investigate the mechanisms underlying this translocation, a hippocampal slice model was developed. Slices were exposed to conditions that simulate ischemic insult, and the subcellular distribution and enzymatic activity of CaMKII were monitored in homogenates prepared after increasing durations of the insult. Semi-quantitative Western blots were utilized by using a monoclonal antibody to the alpha subunit. CaMKII showed that there was a 70% decrease in CaMKII in the post-synaptic fraction and a 150% increase in CaMKII of the pre-synaptic fraction after 20 min of ischemia. Shorter periods of ischemic insult produced a graded change in redistribution. There was little or no change detected in CaMKII in the total homogenates at any time suggesting that little proteinases of CaMKII was evident at up to 20 min in this model. CaMKII activity decreased in the post-synaptic in a parallel fashion with translocation. Activity increased by 20% in the post-synaptic fractions at 5 and 10 min. of ischemia and then decreased back to pre-ischemic levels by 20 min.

We conclude that redistribution of the enzyme occurs in the hippocampal slice model and that this system may be useful in elucidating the mechanisms underlying CaMKII alterations and its impact on ischemia-induced neuronal injury.

Differential Regulation of Multiple Calcium/calmodulin-Dependent Protein Kinase Type II Subunits mRNA's. K.D. Murray*, C.A. Rosasco, and P.J. Jackson Department of Biochemistry and Molecular Biology, Mayo Clinic, Jacksonville, FL 32245

Calcium/calmodulin-dependent protein kinase type II (CaMKII) is a multifunctional enzyme comprising as much as 2% of total brain protein and playing a critical role in excitatory neuronal processing. Originally identified as a multimeric holoheximide composed of α and β subunits, recent cloning analysis has identified two additional subunits, CaMkIβ and γ, as well as a series of alternatively spliced isoforms deriving from these four subunits. Earlier studies have shown that hybridization to CaMKII mRNA is down-regulated throughout hippocampus and neocortex following bilateral ischemic insults and up-regulated when afferent input is removed (Benson et al., 1991, Murray et al, 1993). To investigate whether the other CaMKII subunits undergo a similar regulation we performed analysis using two site hybridization, northern and Southern blots, and ISH on rat brains. The male Sprague-Dawley rats sacrificed at various time-points following the onset of behavioral seizures induced by intraperitoneal injection of kainic acid (K.A., 10mg/kg). The pattern of hybridization to CaMKIIβ,β and β subunit mRNA in saline injected animals was consistent with previous reports in untreated control animals. One notable difference was the presence of low, but detectable hybridization of CaMKII mRNA within the hippocampus. Consistent with the lesion induced seizures, KA treatment produced a stark reduction in hybridization to CaMKII mRNA (90%) throughout the hippocampus and neocortex. Hybridization to CaMKII mRNA was also reduced within hippocampus and neocortex, but to a lesser extent. In contrast, hybridization to CaMKII mRNA was unaffected within hippocampal subfields but dramatically increased throughout the superficial layers of neocortex (5 fold). All mRNA changes were maximal 24 hrs following KA injection. CaMKII mRNA levels were not significantly altered following KA treatment. These observations provide evidence for the regulation of CaMKII at the RNA level and suggest it is more complicated than previously thought.
480.15

In Alzheimer’s disease (AD), the lack of an animal model complicates the analysis of early pathological changes. Mitogen-activated protein (MAP) kinases have been suggested as potential targets for the neurodegeneration in AD. Here, we describe the cloning and molecular characterization of the mouse homolog. Immunocytochemically, MAPK12 identified a cytoplasmic antigen expressed almost exclusively in neurons. The human p46K12 kinase cDNA was used to isolate a 2.5 kb mouse cDNA clone. The deduced amino acid sequence was novel and shared 99% identity with that of the human p46 kinase. Moreover, the 3' untranslated region showed 88% nucleic acid identity with the human cDNA. This suggests a conserved regulatory role for this region. Northern blot analysis showed a 2.7 kb mRNA that was exclusively expressed in the brain and a 2.4 kb mRNA expressed only in testis. All other tissues showed no expression. In situ hybridization showed a similar pattern of expression as that of JF12 antigens. Developmentally, the p46K12 kinase was turned on embryonic day 11 (E11), and was expressed only in post-mitotic neurons. At E17, the mRNA was also present in the dorsal root ganglion as well as neurons in the CNS. These findings are in agreement with the suggested role of ERKs in neuronal differentiation. The distribution of the p46K12 protein along with evidence suggesting its involvement as a pathogenic agent in AD, may lead to development of a possible model for NFT formation.

480.17
THE yl ISOFORM OF PROTEIN PHOSPHATASE 1 IS CONCENTRATED IN DENDRITIC SPINES. C.C. Quiñete*, E.F. da Cruz e Silva* and M.B. Lightbody. Dept. of Pharmacology, Florida State University, Tallahassee, FL 32306.

In vitro hybridization revealed a higher level of expression in dendritic axons than in cell bodies. These results suggest that the yl form of the protein phosphatase 1 may be involved in spine regulation. (Supported by USPHS grant GM06899.)

480.18

Okadate acid (OA) and calyculin A (CaA), specific inhibitors of the protein phosphatase type 1 (PP-1) and 2A (PP-2A), slow the recovery of elevated [Ca++]i caused by K+-depolarization and prolong the divalent cation-dependent action potential in the serotonergic Retzius cells of the leech (Kleinhaus & Zeman, Brain Res. 1993). These studies suggest that inhibition of PP-1 and/or PP-2A may directly modulate the kinetics of the voltage-dependent Ca2+ conductance and/or alter other mechanisms related to Ca2+ movements or buffering.

To identify and quantify the relative amounts of PP-1 and PP-2A found in these neurons protein phosphatase activity. from extracts of leech segmental ganglia and isolated Retzius cells was measured using [32P]orthophosphate as a substrate. The major phosphatase activity in both preparations is similar to the mammalian form of PP-1. The dephosphorylation of phospho-epitopes from extracts of segmental ganglia, isolated Retzius cells and purified mammalian PP-1 was inhibited by OA and by CaA with IC50 values (~50 nM). In addition, enzymatic activity intrinsic to each leech neuronal preparation was inhibited >80% by addition of inhibitor 2, a heatstable inhibitor protein specific for PP-1. That enzyme was also inhibited by calyculin A, but the IC50 values of >20-30 nM is higher than that of the purified mammalian PP-1 and PP-2A. Taken together these results indicate that phosphatase activity in leech nervous tissue is mostly due to an enzyme of the type 1 family.

Studies to characterize the subcellular distribution and composition of this PP-1-like enzyme are in progress.

480.19
CLONING OF PARTIAL cDNAs FOR THREE PROTEIN TYROSINE PHOSPHATASES FROM APlysia CALIFORNICA. F.C. Richardson* and L.K. Kaczmarek. Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06511.

Patch clamp experiments of a voltage-dependent cation channel in Aplysia bag cell neurons have shown that the gating mode and response of the channel to protein kinase A (PKA) are determined by a PKA regulated protein-tyrosine phosphatase (PTPase) (G.T. Wilson and L.K. Kaczmarek, 1993, Nature vol. 366:433-438). As a first step towards understanding this regulatory relationship, a PCR based cloning strategy was employed to isolate potential PTPase isoforms. cDNA was sequenced by reverse transcription cloning, using total RNA isolated from abdominal ganglia and bag cell neurons. This cDNA was then amplified using degenerate primers designed to the conserved catalytic domain of published PTPase sequences. Products of predicted molecular weight were directly ligated into a TA vector and sequenced. Based on protein sequence homology to the catalytic domain of cytoplasmic phosphatases, three clones, named APTPs1, 2, and 3 were isolated. APTPs1 and APTPs2 were 36.3% identical and are most similar to Human PTPs (48.7%) and Rat LAR (67.4%), respectively. APTPs2, which is 38.6% identical to APTPs1 and 36.2% identical to APTPs3, is the most novel of the three isoforms. It's highest similarity to any published PTPase (Human LCA) is only 34.2%. In situ hybridization studies and further cloning will help determine whether any of the APTP isoforms are regulated by PKA in the bag cell neurons.

480.20

I-2 is the regulatory subunit of the ATP-Mg-dependent protein phosphatase, a cytosolic form of type I protein phosphatase (PP-1). Our in situ hybridization study has recently demonstrated that the mRNA for the three catalytic subunits of PP-1 and inhibitor-I(1-1) were differentially distributed in the rat brain (H. Sakagami et al., Mol. Brain Res., in press). However, no information has been available concerning the distribution of the gene expression and the functional significance of I-2 in the brain. We, therefore, cloned cDNA encoding I-2 and determined the distribution of the gene expression for I-2 in the rat brain by in situ hybridization histochemistry. The cDNA encoding I-2 was isolated from a rat brain cDNA library using a Green fluorescent probe for rabbit skeletal muscle I-2 (Park et al., 1994). By in situ hybridization histochemistry using oligonucleotide probe, intense expression signals were detected in the hippocampal pyramidal and dentate regions, as well as in the olfactory bulb. Moderate expression signals were detected in the olfactory neuronal layer, caudate putamen, neocortex, and cerebellar granule cell layer. When compared with this expression pattern with that of I-1 mRNA, several differences were found: the homogenous expression of I-2 mRNA versus the laminar expression of I-1 mRNA in the neocortex; low expression of I-2 mRNA versus high expression of I-1 mRNA in the arachnoid membrane. These findings suggest that the activities controlled by I-2 and I-1 in various brain regions. (Supported by grants 0404020 and 0526023 from the Ministry of Education, Science and Culture of Japan)
CHARACTERIZATION AND REGULATION OF GABAA RECEPTOR SUBUNITS ON NEURONES IN THE HYPOTHALAMIC SUPRAOPTIC (SON) AND PARAVENTRICULAR (PVN) NUCLEI V.S. Fenoloe and A.E. Herbst

1994

461.1 NEUROENDOCRINE REGULATION: MAGNOCYCLULAR SYSTEM

GABA is known to regulate oxytocin (OT) and vasopressin (VP) secretion via the occupancy of GABA receptors. As the different GABA receptor isoforms have different pharmacologic properties, the present study determines the role of making magnocellular neurons and determine whether changes in specific subunit mRNAs are during pregnancy and lactation.

Double labelling studies using antisera specific for either OT or VP and antiserum directed against the b subunit of the GABA receptor were carried out on sections of GABAergic neurons in the SON. GABAergic neurones were confirmed twice, VP-like immunoreactivity was observed in all OT and VP neurones located in the SON express the a2 subunit. In site hybridization experiments using 5S-labelled complementary a subunit, the subunits were confirmed that neurons in the SON expressed a subunit. Silver grain analysis for a and b subunit subunits were carried out on the SON, PVN and corticosterone (CORT), pregnant (day 10 and 19), parturition and lactating rats (day 7 and 14). a mRNA expression in the SON increased during pregnancy, peaking at day 10, and fell in lower levels on the day of parturition. a mRNA expression in the cortex and mRNA expression in SON, PVN and cortex did not show such variations. Furthermore, it appears that expression of an a subunit-containing GABA receptor islet is altered during pregnancy suggesting that GABA influences on OT and/or VP secretion at this time may be determined, in part, by receptor expression.

461.2 OXYTOCIN HORMONE INDUCES PLASTICITY OF OXYTOCIN NEURON ACTIVITY OF SUPRAOPTIC NUCLEUS IN THE FEMALE RAT. T. KITODA, S. YADA and M. NIYATTA, Dept. of Appl. Phys., Kyoto Institute of Technology, Sakyo-ku, Kyoto 606 Japan

It has been demonstrated that oxytocin (OT)-containing neurones in the supraoptic nucleus of rats are facilitated by exogenous oxytocin (OT) and inhibited by removal of virgin females or pregnant females. The present study was carried out to investigate whether OT increases the OT neuron activity in hypothalamic slices prepared from virgin female, pregnant, delivering, lactating, and ovarectomized rats by recording extracellular single-unit activity. Most OT neurones of virgin female and pregnant rats showed inhibitory responses to OT. In contrast, majority of OT neurones were tested in delivering and lactating rats exhibited excitory responses to OT. This excitation reversed to inhibition after the lactating period had ended. OT neurones in over- ecrotomized virgin rats showed excitory excitory responses to OT. This excitation was also reversed to the inhibition by estrogen treatments. It was also found that morphological changes of OT neurones associated with the lactation were unaffected by ovariectomy suggesting that other stimulus such as nipple sucking may be crucial in maintaining the changes. These findings suggest that the oxytocin neurones may play an important role in induction of neural plasticity in rat hypothalamic OT neurones.

461.5 HYPERTONIC SALT-INDUCED CHANGES OF THE OXYTOCIN CONTENT OF THE THORACIC SPINAL CORD: REGULATION BY OPIOD PEPTIDES. V. Neira & J. Hually, Dept. of Biological Sciences, St. John's University, Jamaica, NY 11439

Previous we have demonstrated that oxytocin content of the spinal cord changes in response to various somatosensory stimuli. For example, following immobilization stress of one minute, oxytocin content increases (Brain Res. 137:141, 360) and somatosensory injections of oxytocin content decreases (Life Sci. 53:579-584, 94). The current experiments were designed to determine what factors or factors regulate spinal cord oxytocin content. Spinal cords of rats known to regulate oxytocin release from the neurohypophysis, it was logical to investigate whether spinal cord oxytocin is also under opioid regulation. Male Sprague-Dawley rats were divided into (i) intact control group rats (n=10), (ii) hypothyroidic saline (10% solution), (iii) Naloxone (500 ng/kg BW), (iv) Naloxone + arginine and (v) Naloxone + hypothalamic saline. Injection volume was 0.2 ml/100 g body weight. For Control group, rats were pin-pricked twice, once subcutaneously with Naloxone injection and second time (p), injecting saline (0.2 ml/kg BW). Five minutes after Naloxone injection, the rats were injected with saline and were sacrificed fifteen minutes thereafter. Following organotypic, thoracic part of the spinal cord was isolated from the vertebral column and separated into superficial and ventral half. Oxytocin content of the spinal cord was determined by a specific oxytocin radioimmunoassay. Both oxytocin radioimmunoassay and the step-extraction sample. Our results demonstrate that (i) dorsal half of the spinal cord contains ten fold more oxytocin than that of the ventral half, (ii) both isotonic and hypertonic saline decrease oxytocin content and (iii) Naloxone reduces oxytocin induced decrease of the spinal cord oxytocin content. In conclusion, these results suggest that spinal cord oxytocin is at least partly regulated by opioid peptides.

461.6 INTRACEREBROVENTRICULAR ADMINISTRATION OF INTERLEUKIN-1B SUPPRESSES REFLEX MILK EJECTION IN URETHANE-AESTHETIZED RATS. B.C. Wilson* and A.F. Summerlee*, Dept. of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G-2W1

It has been shown that ivc IL-1B stimulates oxytocin release in male (Neumann et al., 1993, Soc. for Neurosci. Abst. 19(1): 97) and female (Wilson and Summerlee, unpublished data) rats. The IL-1B excites suprachiasmatic neurons (Li et al., 1992, Neuroreport 3: 91-93). Experiments were done using the methodology of Summerlee et al., 1984, Nature 309: 372-374 to investigate the effect of ivc IL-1B on the pattern of reflex milk ejection (RME) in rats at Day 10 of lactation. Urethane-anesthetized rats were implanted with cannulae in the left saphenous vein and a caudal mmamy gland and then placed in a stereotaxic head frame. A microphone was placed with its tip in a lateral cerebral ventricle for ivc injection. Ten rat pups were placed to suck at the nipples of each dam to induce RME. After 6 milk ejections, milk was placed with ivc IL-1B (10 ng/LB PBS-BSA iv) or PBS-BSA (10 ng/LB) and the effect on the pattern of RME observed. IL-1B suppressed RME in 10 out of 16 dams with ivc IL-1B and 0 out of 9 (0%) for those treated with the PBS-BSA vehicle. It is possible that in female rats, IL-1B stimulates milk ejection release while inhibiting suckling-induced ivc injection involved in milk ejection. The data suggest that IL-1B plays a role in mediating suckling-induced neuroendocrine events in the rat.
481.7 INTRACEREBROVENTRICULAR INJECTION OF RELAXIN AFFECTS THE RELEASE OF BOTH VASOPRESSIN AND OXYTOCIN IN ANESTHETIZED RATS. B.J. Geddes and J.L. Summerlee. Dept. of Biomedical Sciences, University of Guelph, Guelph, Ont., CAN, N1G 2W1.

Injections of exogenous relaxin either IV or ICV produce a marked and sustained rise in systemic arterial blood pressure (Parry LJ & Summerlee AJ, 1991). Intravenous relaxin administration has also been shown to substantially affect the release of vasopressin (AVP) and oxytocin (OX) (Way & Leng, 1992; Geddes BJ, Parry LJ & Summerlee AJ, 1994). There is preliminary evidence that ICX relaxin also affects magnocellular peptide secretion (Jones, 1988) so the present study was conducted to make a more thorough examination of the influence of ICX relaxin on vasopressin and oxytocin release. Urethane-anesthetized female SD rats were cannulated such that blood samples (1±mL) and fluid replacement could take place simultaneously. Intraduodenal administration to pregnant rats indicated that the effects of relaxin injected ICV and IV are ostensibly identical which supports the hypothesis that relaxin affects cardiovascular function through a central mechanism. Supported by NSERC Canada and OMAF Canada.

481.8 EFFECT OF OVARIAN STEROIDS ON HYPOTHALAMIC PULSATILE OXYTOCIN RELEASE EVOKED BY SUCKLING IN THE ANESTHETIZED RAT. Q.B. Liang and J. Wilkerson. (SPON: Brain Research) Dept. of Anatomy, Sci. of Medical Sciences, Univ. of Bristol, Bristol, BS8 1TD, U.K.

Effect of ovarian steroids on pulsatile oxytocin (OT) release during suckling was examined in late pregnancy mares by ovariectomy (OX) and steroid replacement. Intramammary pressure recordings of milk-ejection responses were used to detect pulsatile OT release from the magnocellular OT neurons in the hypothalamus. OXV on day 20 of pregnancy significantly increased the frequency of milk-ejection responses on day 22 of pregnancy, compared with sham-OVXed controls (2.0±0.2/min, mean±SE, n=6, P<0.05, Student’s t-test). Replacement with either progesterone (5mg per day, s.c.) or estradiol (5mg per day, s.c.) at 20 min post OVX reduced milk-ejection frequency to the sham-OVXed level. However, these two different steroid regimes caused a remarkable difference in the facilitatory response to intracerebroventricular (i.c.v.) injection of OT. Milk-ejection frequency in progesterone replaced rats was decreased significantly from 2.0±0.2/min before i.c.v. OT to 0±0.2/min after i.c.v. OT (n=6, P<0.05). In OVX oil treated controls, frequency of milk-ejection before and after i.c.v. OT were 3.5±0.2/min and 3.7±0.2/min (n=7). These results suggest that ovarian steroids may play a role in determining the frequency of pulsatile OT release towards the end of pregnancy. Furthermore, the rising level of cortisol and fall of progesterone towards the end of pregnancy may be a necessary prerequisite for allowing the facilitatory action of centrally-released oxytocin on the milk-ejection reflex. Supported by ASRC HG1763.
**481.14**

**VASOPRESSINERGIC INNERTION TO SUBSTANTIA NIGRA (SN) AND VENTRAL TEGMENTAL AREA (VTA) IN THE RAT. T. Yamamoto* and S.T. Kitai, Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

Vasopressin (VP) fibers are widely distributed in the brain in addition to a hypothalamic-hypophyseal system. In addition to classically known antidiuretic and vasoconstrictive actions, VP affects memory process, cardiovascular function, thermoregulation and motor behavior. SN and VTA in the midbrain are known to receive VP innervation, but the cells of origin of these VP fibers are unknown. In this study, we examined the VP innervation to SN and VTA in the rat using a retrograde fluorescence tracing technique and immunohistochemistry. Ten to 14 days following pressure injection of Fluoro-Gold (FG) (2.5 to 4%, 25 to 45 nL) into the SN/VTA region, colchicine (120 µg/kg) was injected into the lateral ventricle. After a 2 day survival period, the rats were transcardially perfused and sacrificed. The brains were removed, post-fixed and stored in phosphate buffer. Using a vibratome 50 µm sections were cut for immunohistochemistry.

The neurons in the bed nucleus of the stria terminals (BST), medial amygdaloid nucleus (MeA), locus coeruleus and paraventricular hypothalamic nucleus (PVN) were retrogradely labeled by FG. In BST there were many double labeled neurons, but there were only a few in the MeA, PVN, and hypothalamic area. These observations indicate that SN and VTA receive VP afferents mainly from BST, but some originate from the hypothalamic area, MeA and PVN.

Supported by NIH Javits Award NS 20702 and the Human Frontier Science Program.

**481.15**


GABA provides most if not all of the inhibitory synaptic input throughout the neuroendocrine hypothalamus. Half of the synaptic boutons are immunoreactive for somatostatin (SOM), paraventricular supraoptic, and arcuate nuclei. Neurons immunopositive for GABA and glutamic acid decarboxylase have been shown in and around SON and other hypothalamic regions, suggesting that inhibitory input to hypothalamic neurons cells might originate predominantly from local GABAergic neurons. Using whole-cell recordings and glutamate microdop in hypothalamic slices, we tested the hypothesis that local GABAergic neurons provide inhibitory input to supraoptic magnocellular neuroendocrine cells. Microdrops of glutamate (20 mM, 50-150 µM in diameter) were applied in the periphery of the SON while frequency and amplitude of postsynaptic currents in supraoptic magnocellular neurons were monitored (n=23). In 4 cells, glutamate microdops dramatically increased frequency and average size of inhibitory postsynaptic currents (IPSCs) while no change in excitatory postsynaptic currents was detected. Glutamate microdrops increased IPSC frequency from 0.5-2 Hz to 3-20 Hz. Glutamate microstimulation also increased the average amplitude in all 4 cells by increasing the proportion of the largest IPSCs, suggesting that more IPSCs were action potential-mediated. These results suggest the hypothesis that GABAergic neurons located in the periphery of SON provide inhibitory input to supraoptic magnocellular neuroendocrine cells.
482.3


Systemic administration of monoclonal interleukin-2 antibodies has been shown to cause selective degeneration of sympathetic preganglionic neurons. In the present study rat paravertebral ganglia were injected i.v. with injection of these antibodies. Exophthalmos, piloerection and ptosis were observed within 1 h after administration. Rats were sacrificed at different time-points, and the adrenal glands were analysed by means of indirect immunohistochemistry and in situ hybridization histochemistry. As soon as 3 h after the antibody treatment a marked increase in the number of chromaffin cells was detectable. Immunocytochemically, respectively, exekalin, calcitonin-gene related peptide, galanin, neurotensin and substance P was seen. At 12 h the peptide mRNA levels were still elevated and there was a concomitant increase in the number of immunoreactive catecholaminergic cells. All these effects were maintained for at least 48 h, however, seventy-seven days after the antibody treatment only exekalin-immunoreactive cells were encountered. A disappearance of acetyleneuropeptide- and exekalin-positive fibres was seen already 3 h after the antibody treatment, and after 24 h no fibres were encountered. In controls, up till 48 h there was no apparent change in the number or intensity of immunofluorescent fibres expressing calcitonin-gene related peptide, galanin, neurotensin or substance P. However, 77 days after the antibody treatment the number of exekalin gene-positive and substance P-immunoreactive fibres was increased as compared to controls. In addition, reappearance of acetyleneuropeptide- and exekalin-immunoreactive fibres was seen at 77 days, although their number still was not comparable to controls. Double-labeling immunohistochemistry revealed that the cells expressing peptides preferentially are adrenergic staining cells. The majority of these cells expressed only one peptide. Both transection of the splanchnic nerve as well as treatment with acetylcholine receptor antagonists mimicked the effects seen after the ACHI-antibody treatment, although changes were less pronounced.

482.5


Paragraphe to investigate the effect of streptozotocin-induced diabetes on concentrations of vitamin A (retinol, retinal, retinoic acid) in the plasma, liver and gonads of rats. To determine any changes in vitamin A metabolism to retinoid enterotoxemia (RE) in hepatic microsomal membranes of rats. To determine any changes in vitamin A metabolism with day-1, day-8 and controls. Diabetes was induced by a single i.v. injection (tail vein) of streptozotocin (55 mg/kg) dissolved in acetic buffer, pH 4.5. Control group received only the vehicle. Plasma glucose concentrations were determined by glucose oxidase method. Both groups were fed ad libitum with a synthetic diet. Animals were sacrificed 42 days post injection. Retinal identity was determined by direct esterification of retinal. Results of the present study suggest that administration of the plasma retinal concentration was significantly lower than those in control rats. However, hepatic retinal concentration of diabetes was significantly higher than that in control rats. The retinoid enterotoxin level in plasma and liver was not notably different between the diabetic and control group. In hepatic tissue, retinoid concentration was significantly increased in comparison to control group while retinal level was not perturbed. After hepatic, hepatic retinoid concentration of diabetes was significantly higher than that in control rats. The retinoid enterotoxin level in plasma and liver was not notably different between the diabetic and control group. In hepatic tissue, retinoid concentration was significantly increased in comparison to control group while retinal level was not perturbed.

482.6


The mechanisms underlying the decrease in drug requirements for central and peripheral anesthesia during pregnancy in humans are not yet understood. Sex hormone effects on excitable membranes have been suggested as a possible cause. Experimental pregestation can be used to examine the drug effects in rats. The results of this study suggest that the present study examined the developmental time course of losartan-induced PRA stimulation during the period in which functional sympathetic control of the renin-angiotensin system emerges. Neurotensive Water-Kyoto (WKY) and spontaneously hypertensive (SHR) rats were used as animal models to study this phenomenon. 2) The possibility of altered pharmacokinetics of LA in the gravid rat by studying lidocaine uptake in a peripheral nerve preparation. Pregnan (P, n=3) and non pregnant (NP, n=5) rats were briefly anesthetized with 10% chloral hydrate. A unilateral sciatic nerve was performed in a dose of 0.1 ml of 1%labelled lidocaine (%LD). After recovery from general anesthesia (1-2min), proprioception, motor function and responses to superficial (sking pinch) and deep (knee pinch) tests were tested. As soon as deep pain sensation returned, the animal was sacrificed. The sciatic nerve rapidly removed, frozen, cut in 5 mm segments, dehydrated, weighted, digested and the uptake of *LD measured by liquid scintillation counting of the different segments. A significant difference in the time for return of deep pain sensation was observed between the 2 groups (P<0.05). The fraction of the total *LD injected dose found in the nerves was 0.49±2.4% for the NP rats and 0.80±0.08% for the P rats. *LD distribution along the nerve was investigated in the two groups using the 2-gamut test (2.5 mm) and resulted in a significant difference between the two groups. In summary, the duration of anesthesia was prolonged in rat rats as was observed in humans, but the amount of *LD in the nerve was the same in both groups. This effect might be due to an increase in uptake of LA (e.g., due to an increase in nerve sheath permeability) 2) a decrease in clearance of the LA from the nerve or 3) both.

482.7

ELECTROPHYSIOLOGICAL EFFECTS OF NORADRENERGIC AGONISTS ON NEURONAL AREAS OF THE STRIATUM AND CAUDAL MEDULLARY ORIGINS OF THE CATECHOLAMINERGIC INNERVATION M.G. Terenzi and D. Ingram Department of Anatomy, School of Medicine, University of British Columbia, Vancouver, BC, V6T 1Z2, Canada.

The bed nucleus of the stria terminalis (BST) is a limbic collection of neurons at the junction of the hypothalamus and septal area. The BST are important for autonomic functions and regulate activity associated with maternal behaviour and the retraction reflex. The BST are innervated by medioventral noradrenergic groups (A1, A2 and A6) and high turnover of noradrenaline occurs in the BST during suckling. We investigated the role of catecholamines from the A1/A2 groups to the BST using fluorescent retrograde tracing and tyrosine hydroxylase (TH) immunocytochemistry. The results showed that the projection from the caudal medulla originates in the lateral medulla and the nucleus of the rassus cuneiformis (NTS). Most of the input is ipsilateral and terminates in the BST ventral to the anterior commissure. Almost no labelling is observed in the caudal medulla when the injection was in the caudal BST. TH immunocytochemically retrogradely labelled neurons were observed in A1/C2 and A2/C2 and a projection of noradrenergic neurons was observed in the NTS in the midline, dorsalateral to the central canal. Electrophysiological studies showed that the basal firing of neurons in the BST is inhibited by iontophoresis of norepinephrine and by noradrenergic antagonists such as clonidine, neuronal activity increases, and the neuronal activity increases, and the neuronal activity is blocked by phenylthio. These neurons were also inhibited by electrical stimulation of the A2NTS area in vivo. The inhibition resulting from iontophoresis of clonidine and the electrical stimulation of the BST is blocked by iontophoresis of the alpha-2 antagonist yohimbine. The BST receive strong catecholaminergic innervation from the medulla which appears to exert an inhibitory effect via alpha-2 receptors and an excitatory effect via alpha-1 receptors.

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482.8

INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) PROTECTS GT-7 CELLS FROM OXIDATIVE INJURY. M.A. Sottini*, L. Scapagnini and P.L. Canico Instituto of Pharmacology, University of Catania School of Medicine and "Chair of Pharmacology, University of Pavia School of Dentistry, Italy.

The neuroprotective action of insulin-like growth factor-1 (IGF-1) has been recently demonstrated in models of cerebral and retinal degeneration. Immortalized hypothalamic, gonadotroph-releasing hormone- (GnRH) secreting, GT-7 cells display a marked sensitivity to toxicity induced by reduced glutathione- (GSH) depleting agents that in these cells cause necrotic death. GT-7 cells express IGF-1 receptors whose activation causes a bidirectional modulation of GSH secretion. On these bases we have studied the potential protective effect of IGF-1 in GT-7 cells. Subconfluent GT-7 neurons cultured for two days were exposed to different concentrations (50-500 uM) of buthionine sulfoximine (BSO), which reduces the intracellular levels of GSH, for 24 hours or to dibutyryl cyclic AMP (DBcAMP, 1 mM) which increases the levels of GSH, for 1 to 3 hours. Both treatments produced extensive neuronal death that was more pronounced in DEM-treated cells. Concomitant exposure of GT-7 cells to IGF-1 and the mammalian growth factor resulted in a remarkable reduction of neuronal death as assessed either by trypan blue exclusion or by staining with fluorescein diacetate and propidium iodide. The neuroprotective effect of IGF-1 has been compared to that induced by the potent antioxidant idebenone that, in this model, provided a virtually complete protection of the oxidative stress produced by either BSO or DEM. These results suggest that IGF-1, as already proposed in different cellular systems for various growth factors, may exert its neuroprotective action through antioxidant mechanisms.

Egglaying behavior in mature Aplysia occurs in response to egg-laying hormone (ELH) released from neuroendocrine bag cells; ELH release is triggered by a prolonged pattern of axonal-neuronal firing (afterdischarges). Electrical stimulation of bag cells from immature Aplysia does not trigger afterdischarges. However, prolonged depolarization (lag phase potentials) can be elicited in the presence of the K+ channel blocker TEA (Nick et al., Soc Neurosci, Abst 708, 1993). In the present study, we sought to determine whether stimuli that might cause firing of bag cells that are incapable of showing afterdischarges contain the functional mechanisms required for secretion. Sucrose electrodes were used for both electrical stimulation and extracellular recording from bag-cell preparations (avg body wt=256 g) or immature (avg body wt=10 g) Aplysia. Medium bathing the preparations was collected every 5 min before, during, and after stimulation for a total of 125 min. Samples were frozen at -20 °C until radioimmunoassay for ELH was performed. Immature preparations (n=3) showed -9 plateau potentials (no afterdischarges), while mature preparations (n=3) showed afterdischarges lasting -18 min with ~650 action potentials fired during that period. Both immature and mature bag-cell preparations secreted ELH above baseline, although the total amount (~36 ng/ml vs. -2400 ng/ml) and duration (-25 min vs. 105 min) of secretion was significantly lower from immature preparations. These results indicate that immature bag cells that do not show afterdischarges can nonetheless be stimulated to release ELH, and therefore must contain the necessary cellular machinery for secretion. Supported by NIH HD 28336 (to NSF), NSP-BNS 614681 (to TC) and NIH-N5 18492 (to LLK).

482.11 EFFECTS OF STREPTOZOTOCIN-INDUCED DIABETES ON NADPH-DIPHOSPHATE POSITIVE NEURONES IN THE RAT PANCREAS. S.B. Try*, G. Burnstock*, Department of Anatomy, National University of Singapore, Kent Ridge, Singapore 0511 & Department of Anatomy and Developmental Biology, University College, London, WC1E 6BT, U.K.

In streptozotocin (STZ) rats, NADPH-diphosphatase positive neurones were dispersed in the interlobular septa, near blood vessels and ducts as well as within pancreatic lobules. This neurone activity was localized in the cell cytoplasm but not in the nucleus. Most of the neurones were heavily labelled while others were moderately to lightly and some were negative. Some labelled neurones sent processes that ramified amongst the exocrine acinar cells. Numerous fine varicose NADPH-diphosphatase positive fibre nerves formed networks around the individual islet cells. At 8 weeks post-diabetes, the number of NADPH-diphosphatase positive neurones and nerve fibres was reduced. Labelled neurones that persisted were solitary or in small groups (2-5 cells). They were heavily labelled for NADPH-diphosphatase activity, some appeared rounded and possessed few short and stubby processes. Lumps of langerhans were not easily distinguishable and their associated nerve networks seemed to have disappeared. NADPH-diphosphatase positive macrophages were also found in the interlobular spaces. These results suggest that diabetes has a drastic effect on NADPH-diphosphatase positive neurones and nerve fibres in the rat pancreas.

482.13 DECREASED GLUCOSE, BUT NOT FATTY ACID AVAILABILITY INCREASES FOS-LIKE IMMUNOREACTIVITY IN THE CAUDAL BRAIN STEM OF FEMALE SYRIAN HAMSTERS. B.F. Florey*, Y. Zhu, and J.E. Schneider. Department of Psychology, Lehigh University, Bethlehem, PA 18015

In Syrian hamsters, extrudary cycles are inhibited by food deprivation or treatment with 2-deoxy-d-glucose (2DG), a drug that inhibits glucose metabolism, and methyly-palmitate (MP), a drug that inhibits fatty acid oxidation. For example, hamsters become anorexic when treated with high doses of 2DG alone (1750 mg/kg), or with low doses of 2DG (500 mg/kg) and MP (20 mg/kg, respectively), but not with either drug alone at low doses. Systemic administration of 2DG at high doses (<1750 mg/kg) stimulates expression of the proto-oncogene c-fos in the area postrema and in the nucleus of the solitary tract (NTS). These and other data suggest that the effects of 2DG on extrudary cycles may occur via a gliaocpic signal detected in the APNTS. The present study investigated whether other metabolic changes also affect c-fos expression and general activation in the APNTS. Hamsters received one of the following treatments: (1) 1750 mg/kg (high dose) (2) 750 mg/kg 2DG (low dose) (3) 20 mg/kg of MP (low dose) (4) low doses of both 2DG and MP (5) high dose vehicle alone. c-fos was observed in the APNTS only in groups that were treated with high doses of 2DG. This suggests that glucose deprivation (decreased glucose availability) but not lipoprivation (decreased availability of metabolic fuels necessary for reproduction may be specifically related to glucose availability. MP may have effects on carbohydrate and/or lipid metabolic effects via the APNTS. Supported by research grant BNS9212056 from NSF.

482.10 VARIATIONS OF MEMBRANE CURRENTS DURING SOMATOSTATINE RELEASE AS MEASURED ON IDENTIFIED HELIX NEURONS BY AN IMMUNOMOLECULAR TECHNIQUE. MATH P., DABRON P., CRIST M., BRIDGM & GOLAM. Neuroendrobiology du développement,25030, Besançon, France and Neurobiologipe Moluinaire, C08,13000 Marseille, France.

Helix neurones of the visceral ganglia were studied in-situ using simultaneous current clamp and somatostatin release using a micro-immunoelectrode. The results have shown it exists a somatostatin release in the extracellular spaces. This release was potential-dependent since it appears for mature bag cells (lag phase potentials) but it was present at +20 mV. Nevertheless, the release intensity seemed to voltage-independent but was delayed when membrane potential increase. Calcium channels blockade suppressed the release. Under slow stimulations (10 hz, +20 mV) the release became cyclic during some minutes but higher frequency increased the somatostatin release in a dependent manner. Even-though a recent demonstration of a noticeable regional difference of calcium channels density, it seemed that somatic eosyoxin characteristics were a good fit for synaptic release. This is in good agreement with previous works which have demonstrated that eosyoxin was not only related to calcium channels but also to sodium channels.


There is evidence on the existence of different ovulatory thyrhythmia between the right and left ovary (RO, LO) depending on ovarian innervation; which is very significant in hemivariectomized [Novx] animals. Present study was conducted to analyze if the blockade of ovarian innervation induced by lidocaine-adrenaline (LiA) injection into the ovarian bursa, previous the extravasation of controlateral ovary, affects the ovulatory ability by the in situ ovary, performed at 15.00 h in the day of proestrus of 4-day cyclic rats. The animals were sacrificed on the next expected day of estrus. When LiA was injected to the LO and the LO extraptipated 0/6 rats ovulated, while when the RO was injected and the LO extraptipated 8/8 rats ovulated (p<0.01). When Novx by the LO or RO was performed without LiA injection, 8/8 and 9/9 rats ovulated. 6/6 rats treated at 18.00h with LiA on the LO and the RO extraptipated ovulated next morning.

Present results support the hypothesis that the RO is more dependent on neural information than the right one. Supported by DGAPA grant IN210893 and PIII.


Inflammation produced by complete Freund's adjuvant injection in the rat hindpaw produces plasma extravasation, and histamine and inflammatory mediator, bradykinin (160 nM), perfused through the knee joint. This negative feedback process has been shown to be neurally mediated with capacin (100 mg/kg) to deplete most of their unregulated primary fibers. Injection of the hindpaw also inhibited bradykinin-induced plasma extravasation in the knee joint. Acute surgical interuptions of lumbar sympathetic affereances to the hind limb did not attenuate the depression of bradykinin-induced plasma extravasation produced by c-fos injection. Injection of bradykinin into the hindpaw did not depress negative feedback process is mediated by activation of primary afferent C fibers. Elevation of C-fibers with electrical stimulation (25mA, 3Hz) of the hindpaw also inhibited bradykinin-induced plasma extravasation in the knee joint.

Acute surgical interuptions of lumbar sympathetic affereances to the hind limb did not attenuate the depression of bradykinin-induced plasma extravasation produced by c-fos injection. Injection of bradykinin into the hindpaw did not depress negative feedback process is mediated by activation of primary afferent C fibers. Elevation of C-fibers with electrical stimulation (25mA, 3Hz) of the hindpaw also inhibited bradykinin-induced plasma extravasation in the knee joint.

Our data demonstrate a negative feedback inhibition of an inflammatory process, which is mediated by sympathetic and peripheral afferents. It is mediated by ascending pathways in the spinal cord, and probably the hypothalamic-pituitary-adrenocortical axis. Supported by NIH grant AM32634
AFFERENT PROJECTIONS TO THE ROSTRAL NUCLEUS RAPHE PALLIDUS BY IONTOPHORETIC APPLICATION OF CHOLERA TOXIN B.


In order to investigate its role in thermoregulation, the afferents to the rostral nucleus raphe pallidus (nRNP) were examined by extremely restricted injection sites of cholera toxin b, which seemed necessary, since the nRNP is wrapped both dorsally and laterally by the central nucleus raphe magnus (nNRM).

Substantial bilateral afferents were received from the precentral, the medial infralimbic, and the subpericaleral cortex for the nRNP.

Strong afferents from the periventricular preoptic area, the paraventricular and the dorsal hypothalamic area could be confirmed in this study; additional afferents from the caudal preoptic area and the adjacent bed nucleus, the central amygdala and the perifornical lateral hypothalamic area were shown. Compared with the nNRM, fewer were afferents from the fields of Forel, the midbrain and pontine periaqueductal gray and the deep mesencephalic reticular formation.

Specific afferents for nRNP, but not for nNRM, were found close to the dorsal raphe nucleus in the ventromedial pontine periaqueductal gray and laterally to the third nucleus inside the midbrain reticular formation. Afferents from the nucleus Kolliker-Fuse and the lateral medullary reticular formation were stronger for nRNP than nNRM, afferents from the subarcuate nucleus, the lateral parabrachial area and the caudal pontine reticular formation less intense for nRNP than nNRM. By highly restricted injection sites, substantial afferents could be shown for nRNP from key structures of central nervous thermoregulation.

PREOPTIC PROSTAGLANDIN E2 (PGE2) AND CORE TEMPERATURE (Tc) RESPONSES OF GUINEA PIGS TO LPS i.v., INDOMETHACIN i.m., AND/OR PGE2 i.a. ADMINISTRATION. E. Schin, M. Erykowski, L.J. Unger and C.M. Blintz*. Dept. of Physiology & Biophysics, Univ. of TN, Memphis, TN 38163.

PGE2 has been postulated to be a central mediator of fever. It is generally believed that PGE2 is produced in the preoptic area (POA) because its level increases both in the third ventricle and the POA in response to i.v. pyrogen. However, lately the question has arisen whether PGE2 may in fact be formed outside the brain substance and then diffuse into it across cerebral vessels. If produced outside the brain substance, the blockade of its synthesis should prevent LPS-induced fever, whereas the intracarotid infusion of PGE2 should produce an increase in Tc as well as in preoptic PGE2. To verify this hypothesis, continuous measurements of PGE2 levels in the POA were made (sampling by microdialysis and analysis by RIA). Tc was monitored continuously during the following experiments: 1) Following a 90-min stabilization period, indomethacin (50 or 50 mg/kg) was administered i.m. 30 min before S. enteritidis LPS (2 µg/kg, i.v.); 2) PGE2 (100 or 10 µg/kg) was infused at 2 µl/min into a carotid artery for one hour. LPS-induced a temperature of 1.2°C was consistently associated with an increase in the level of PGE2 in the POA. Indomethacin at 10 mg/kg attenuated the LPS-induced fever and prevented the associated increase in PGE2 for 90 min after fever onset; moreover, PGE2 was significantly reduced by comparison to controls. Indomethacin at 50 mg/kg completely abolished both the fever and the increase in preoptic PGE2. Intracarotid infusion of PGE2 produced a dose-dependent hypothermia without any increase in preoptic PGE2. The blockade of fever and inhibition of the increase in preoptic PGE2 levels further substantiate the presumptive link between preoptic PGE2 and fever. The failure of exogenous PGE2 intracarotid infusion to induce increases in Tc and preoptic PGE2 excludes the possibility that PGE2 formed outside of the brain diffuses into the POA across cerebral vessels and induces fever. (Supported by NIH grant NS 22716.)

BOMBESIN-INDUCED HYPOTHERMIA IN ADRENALONIZED RATS. C. Barten*, D.A. York and G.A. Bray. Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA 70808.

Bombesin (BOM) is a tetrapeptide known to produce hypothermia when administered centrally in rats tested at normal ambient temperatures. Previous studies have demonstrated bombesin-induced hypothermia to require a state of reduced sympathetic nervous system activity. The importance of adrenal activity on this phenomenon is unknown. The present study examines the impact of bombesin injection (100µg/50µl) or vehicle (VEH) into the lateral ventricles on core body temperature of food-deprived (24hr) adrenalectomized (ADX) and sham operated (SADD) rats with peripheral corticosterone (CORT) replacement (0.1 mg/kg, i.p.) or vehicle (VEH). Blood pressure and heart rate were monitored in ADX rats as assessed by serum aldosterone assay at the completion of testing. This produced a significant difference between those rats exposed to the four experimental conditions: VEH/VEH/ADX, VEH/SADD, BOM/VEH/ADX, & BOM/CORT/ADX (p<0.03). Conditions were recorded at time of central injection and 60 min later. Central bombesin injection in all conditions as compared to central vehicle with a significantly greater hypothermia seen in ADX + CORT (+4.1±3.9°C) & VEH + CORT (+10.1±8.5°C) vs ADX (1.9±3.2°C) + VEH (+1.9±3.2°C).

These results demonstrate that adrenalectomy potentiates bombesin-induced hypothermia, and suggests that corticotrophin-releasing hormone and adrenal catecholamines modulate this phenomenon.
Efferent sympathetic nervous activity (SNA) from a small branch of the intercostal nerve, which ended in the intercostal brown adipose tissue (IBAT), and metabolic heat production (MHP) were measured in C57BL/6J male adult and aged mice under urethane/isoflurane anesthesia and was recorded as HR variability, from the recordings in the absence of urethane and isoflurane anesthetics and an IC1 blocker. HR variability was calculated on the basis of arterial pressure variability measured in the urethane- and isoflurane-free state and was expressed as the difference between the mean values of the heart rate standard deviation before and after the application of the specific blocker.

The studies were conducted in two experimental groups, one consisting of adult mice and the other of aged mice. Both groups were divided into two subgroups, one receiving the IC1 blocker alone and the other receiving the IC1 blocker plus the alpha2-adrenoreceptor agonist clonidine. The results obtained from the adult mice were compared with those from the aged mice to determine whether age-related changes in HR variability occurred.

In conclusion, the present study provides new insights into the role of SNA and MHP in regulating HR variability in mice. The findings suggest that age-related changes in HR variability are associated with changes in SNA and MHP, and that these changes may be mediated by alpha2-adrenoreceptor agonists. Further studies are needed to investigate the underlying mechanisms and their potential therapeutic implications.

The activity of preganglionic sympathetic neurons is maintained by an excitatory input from neurons located in the RLVM. Previous studies conducted in tissue slices (Sien et al. Brain Res. 1988; 451:345; and 1991, 556:61) examined the morphology and presence of catecholamine containing RLVM neurons. However, due to functional and anatomical heterogeneity in this medullary region, it is important to examine neurons which can be directly compared as baroreceptive and reticular neurons. This was possible in the present experiments conducted in vivo, in pentothal-anesthetized adult rats (see Lipinski et al., this meeting). Recordings were made with microelectrodes filled with 1% Neurobiotin or 3% Lucifer Yellow. RLVM neurons which responded with IPSPs following stimuli applied to the aortic nerve were intracellularly labeled. Some of these neurons could be excited antidromically following stimulation in T2 segment. Filled somas were found ventromedial to the compact formation of nucleus ambiguous. Dendrites extended up to 800 μm from cell bodies. Some main axon bifurcated in the dorsomedial tegmentum, and a few had axon collaterals which displayed bouton-like varicosities in the ipsilateral RVLM. CV adrenergic cells were revealed by immunofluorescence using antibody to tyrosine hydroxylase. Some injected neurons were shown to be double labeled.


RLVM bulbo spinal neurons were identified by the presence of retrogradely transported green latex microspheres injected under anesthesia in the spinal cord at Ts, 4-7 days prior to sacrifice. Then these cells were labeled intracellularly in fixed 100 μm-thick sections (horizontal or parasagittal) with Neurobiotin that was visualized with an avidin-HRP reaction. Drawings and quantitative analysis of morphological features were done with the Neuronal software. Some of the sections were doubly labeled for tyrosine hydroxylase (TH) and Neurobiotin using fluorescent tags.

In coronal sections, 83 well-filled cells were analyzed. They had 2 to 11 dendrites (mean ± SD, 3.2 ± 1.2) that extended 212 ± 10 μm (up to 690 μm) and branched up to five times. Their perikarya were fusiform (66%), round (16%), triangular (10%) or multipolar (8%) and had a mean cross sectional area of 417 ± 15 μm², corresponding to a roughly computed diameter of 23 μm (range: 14 to 31 μm).

Comparing parasagittal (23 cells injected), horizontal (N=21), and coronal (N=83) sections, we failed to find a predominant orientation in any plane for the extension of the dendritic tree in the whole population of the injected RVLM cells.

Forty five percent of the RVLM neurons examined in the coronal plane (37/83) had sparsely distributed spines and protruberances on their perikaryons and dendrites. In double stained cases, TH-positive cells, which account for 67% of the injected retrogradely labeled RVLM cells, contained both neurons with and without spines. (Grant: HL 26705 to PGG and NS 17143 to GFA).

GABA AND GLUTAMATE INPUTS TO BULBOSPINAL NEURONS IN RAT ROstral VENTROLATERAL MEDULLA. I.J. Llewellyn-Smith*, J.B. Minson, P.M. Plojesky, L.F. Amolda and J.P. Chalmers. Department of Medicine and Centre for Neuroscience, Flinders University, Bedford Park, SA, 5042, AUSTRALIA.

A potent vasopressor area coincides with the C1 group of catecholaminergic neurons in the rat rostral ventrolateral medulla (RLVM). Bulbo spinal RLVM neurons participate in the baroreflex and receive anafferent inhibitory input from the caudal ventrolateral medulla and an afferent excitatory input from the nucleus tractus solitarius. These pathways probably use amino acid neurotransmitters.

To study amino acid inputs in synapses on bulbospinal RLVM neurons, we injected cholera toxin B-gold into the intermedio lateral column. Bulbo spinal neurons were visualized with silver-intensification. Immunoreactivity from neurons containing glutamic acid was detected by post-embedding immunocytochemistry; and GABA or glutamate, by postembedding immunogold. Only the inputs to bulbospinal RLVM neurons forming a light cluster ventral to the nucleus ambiguous complex were examined. The cell bodies and dendrites of these neurons received synapses and contacts from GABA and glutamate nerve fibers. GABA occurred in 43% (23/54) and glutamate in 73% (39/54) of inputs. GABA was TH neuron in 39% (59/150) and glutamate in 54% (74/136) of inputs to non-TH bulbospinal neurons. Studies are underway to determine the origin of the amino acid synapses on bulbospinal RLVM neurons and whether GABA and glutamate are co-localized in any of these inputs.

EFFECTS OF GAAB RECEPTOR AGONIST BACLOFEN ON NEURONS IN ROstral VENTROLATERAL MEDULLA (RLVM): AN "IN VITRO" AND "IN VIVO" ELECTROPHYSIOLOGY STUDY. Y.-W. Lee and P.G. Gugenheim. Department of Pharmacology, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

We studied the effects of baclofen, a GABA receptor agonist, on neurons in the RLVM of the rat. In 17 neurons recorded intracellularly with sharp electrodes, bath application of baclofen (1-3 μM) produced a membrane hyperpolarization of 812 ± 160 mV (baseline 582 ± 160 mV, n=7), 2 a 15% decrease in the membrane input resistance (baseline 138±17MΩ, n=10), 3 a decrease in firing rate (n=14) and 4 a decrease in spontaneous post-synaptic potentials (n=10). Of 83 single units recorded extracellularly in the RLVM in vivo, all but 2 were inhibited by baclofen (1-1.3 μM). The inhibition was concentration dependent (0.1-3.0 μM, max. inh. = 94±8%, n=16), and persisted in low-Ca²⁺/high-Mg²⁺ medium (n=15). The GABAB receptor antagonist CGP35485A (1 μM, n=10) and CGP35485A (1 μM, n=15) or 2-hydroxysaclofen (0.5 mM, n=3), attenuated the inhibitory effect of baclofen (1-3 μM), but not that of GABA or muscimol (GAAB receptor agonist). In vivo, iontophoresis of baclofen (40-120 nA) inhibited most RLVM neurons including 13 out of 16 bulbospinal baroexcitatory cells. This effect was antagonized by iontophoresis of the GABAB antagonist, CGP35485A (60-80 nA, n=6), but not the GABAB antagonist, bicuculline (40 nA, n=5). These results suggest that 1) most RLVM neurons including sympathetically premotor neurons have somatodendritic GAAB receptors, 2) GAAB receptors in the RVLM may play a role in control regulation of cardiovascular function.
484.11 DIFFERENTIAL TIME- AND DOSE-RELATED EFFECTS OF HEMORRHAGE ON TYROSINE HYDROXYLASE (TH) AND NEUROPEPTIDE Y (NPY) mRNA LEVELS IN MEDULLARY CATECHOLAMINERGIC NEURONS. R. W. K. Chua* and P. E. Suschek. The Sak Institute, La Jolla, CA 92037.

Hypotensive hemorrhage induces cellular activation and up-regulation of TH mRNA in medullary catecholaminergic cell groups. To shed light on the significance of the widespread up-regulation of NPY in amine-secreting neurons, quantitative hybridization histochemical methods were used to compare the impact of graded doses of hemorrhage (15, 20 and 25%) on the time course of changes in relative levels of TH and NPY with respect to baseline staining for nucleolar, autonomic and other organ distribution in the brain. The data permitted comparisons between cells that ostensibly were not targeted by the challenge.

The major results can be summarized as follows: (1) Hemorrhage-induced significant up-regulation of TH mRNA in all medullary amine cell groups and NPY transcripts in all but the A2 region. At later time points (2-4 hrs) these changes were predominantly seen in Fox-iir neurons. (2) Neuronal cell groups displayed more robust maximal increases in relative levels of TH mRNA, while the reverse was true of the adrenergic cell groups. (3) Hemorrhage-induced up-regulation of TH and NPY mRNAs in Fox-iir neurons displayed differential time courses, with NPY responses peaking more rapidly, particularly in the C1 and C2 adrenergic cell groups. (4) At early time points (0.5-1.0 hr), a majority cells displaying increased levels of each transcript in the aminergic regions of interest were non-Fox-i. Sampling at early time points, however, may preclude the ability of cells to mount a detectable Fox protein response. These findings indicate that hemorrhage differentially affects relative levels of TH and NPY mRNA in medullary catecholaminergic cell groups that have no prior participation in anesthetic-mediated atropine and/or autonomic control mechanisms. The surprisingly robust NPY mRNA responses in adrenergic cell groups suggest a mechanism by which the peptide content of the projection fields of these cell groups is defended.


Spike-triggered averaging and coherence analysis was used to study the relationship between the discharges of 246 CVLM neurons and the 10-Hz rhythm in renal sympathetic efferent nerve fibers (SN) in urethane-anesthetized cats. The study was repeated after bilateral microinfusions of muscimol at four sites in the CVLM ipsilateral to the renal nerve recording in chemically inactivated neurons in this region. This procedure reversibly eliminated the 10-Hz rhythm in SN. Thus, we propose that CVLM neurons are elements of the network responsible for the 10-Hz rhythm in SN. (Supported by NIH grants HL-33366 and HL-13187.)


We previously demonstrated directional relationships among the 10-Hz rhythmic discharges of the cardiac, renal, and spriophageal sympathetic nerves by using partial coherence analysis in baroreceptor-denervated, urethane-anesthetized cats (Simpson et al., Soc. Neurosci. Abstr. vol 19, Part 1, 1993). Partialization mathematically removes the portion of the coherence of two signals attributable to the sources of a third signal. For example, we found partialization using spriophageal sympathetic nerve discharge data that reduced the coherence of the 10-Hz discharges of the left and right cardiac nerves to a significantly greater extent than did partialization using renal SN. Such results may reflect, among other things, 1) nonuniform coupling of multiple brain stem oscillators, each controlling a different nerve or 2) nonuniform crossstalk among spinal circuits that control different nerves but share inputs from a common source. In the present study, we compared coherence estimates and partial correlation between the left and right cardiac nerves in the presence of vagal parasympathetic nervous system activity in response to artificial means respiratory pattern and low level right renal nerve stimulation in urethane-anesthetized cats. The results indicate that the coherence of these signals is reduced by vagal parasympathetic nervous system activity and is abolished in a stochastic manner. The results suggest that a period of reorganization within the physiological range of rhythmic generating circuitry (Supported by NIH grants HL-28931 and HL-20864.)


We examined sympathetic nerve activity (SNA) and exaggerated sympathetic responses to isometric contraction in the spontaneously hypertensive rat (SHR) suggest that central nervous system dysfunction may contribute to the pathogenesis of hypertension in this model. In the present study, we compared the hypertensive responses of vasopressor neurons in the caudal ventrolateral medulla (CVLM) is diminished in SHR. Prior studies have demonstrated that excitolesions in CVLM in Sprague Dawley rats increase both SNA and mean arterial pressure (MAP). In urethane-anesthetized SHR and Wistar-Kyoto (WKY) rats, we examined the effects of kainic acid lesions in CVLM on spontaneous SNA, resting MAP and the evoked nerve responses to POH stimulation. In all rats, CVLM lesions produced significant increases in both SNA and MAP. Peak post-lesion MAP was no longer significantly different between strains (140 ± 30 mm Hg SHR vs. 129 ± 30 mm Hg WKY). However, the increase in total power (RMS) in SNA after the lesions was significantly smaller in SHR (111 ± 14) than in WKY (203 ± 30; P < 0.01). In both strains, post-lesion evoked responses to POH stimulation were greater than control, although this enhancement of evoked sympathetic responses was smaller in SHR than in WKY. We conclude that CVLM neurons may be less active in SHR, resulting in diminished sympathoexcitation.


A 10-Hz rhythm is ubiquitous in different species and conditions, and has been studied within cardiovascular reflexes in the presence of different targets in urethane-anesthetized or decerebrate cats. This rhythm can coexist with a low frequency component (a cardiac-related rhythm in cats with intact baroreceptor nerves). This rhythm is generated by a partialization of a fundamental oscillation with a period near 2-6 Hz (baroreceptor denervation). Spike-triggered averaging was used to study the relationship between the discharges of individual medullary neuronal groups and SN. Data from these studies show that the generators of the 10-Hz rhythm and low frequency components in SN are comprised of separate pools of brainstem neurons. First, in baroreceptor-denervated cats, medullary sympatetic nerve cell groups with activity correlated to the 10-Hz rhythm did not have activity correlated to the irregular 2-6 Hz oscillations. Second, in baroreceptor-innervated cats, CVLM nerve cell groups with activity correlated to the 10-Hz rhythm did not have discharge correlations 1:1 to the cardiac-related rhythm when both rhythms were prominent in the spectra of SN. Third, for CVLM neuron activity with activity correlated to the 10-Hz rhythm, the relationship between their discharge and SN was eliminated when the predominant pattern in SN was changed to a cardiac-related rhythm by raising arterial pressure. Fourth, lateral tegmental field neuronal activity had activity correlated to the low frequency components but not to the 10-Hz rhythm in SN. Some rostral ventrolateral medullary and raphe neuronal groups had activity correlated to both the 10-Hz rhythm and low frequency components in SN. Thus neurons in these regions receive converging inputs from the two generators. (Supported by NIH grants HL-33366 and HL-13187.)


Simultaneous recordings of cervical SYMP, splancnic and parovascular (PHR) activity along with arterial and directly recorded atrial EKG and end-tidal CO_2 were obtained in Saffan-anesthetized, paralyzed and artificially ventilated (100% O_2) 1-4 days old kittens. Power spectra and coherence estimates between SYMP nerves and between SYMP and PHR discharge were obtained by a FFT routine. PHR discharge defined the inspiratory (I) and expiratory (E) epochs used for gating spectral estimates. Coherence between SYMP nerves was significant at birth in the 3-5 Hz range; the value of coherence was not age-related. In the 5-12 Hz range, coherence revealed age-related maturation attaining significance circa 2 wks. At about 16 days, higher frequencies (>12 Hz) were present in the preganglionic SYMP power spectra. Highest coherence estimates related to low frequencies were significant until about 4 wks. In both power spectra and coherence estimates, these higher frequencies disappeared after 4 wks of age. Coherence between PHR and SYMP was not significant until circa 3 wks. By 1 mo, SYMP activity resembled that observed in adult mammals (Gootman & Cohen, Acta Physiol. Polon. 1973, 24-97). The results suggest that there is a period of reorganization within SYMP rhythm generating circuits. (Supported by NIH grants HD-31641 and HL-28931.)
CARDIOVASCULAR ANATOMY, MEDIUM-SIZED BY VALUES OF...  

We have previously shown by chemical microinjection methods (Brain Research, 548:279-286, 1991) that the rostral depressor area (RDA) of the brain stem in cat contains sympathoinhibitory neurons involved in cardiovascular regulation. We now report on pentobarbital anesthetized cats a study of 55 cells in the RDA whose spontaneous activities, detected extracellularly with a metal micro-electrode, were found to be correlated with tonic activity in 2-6 Hz sympathetic discharge of the inferior cardiac nerve. The method employed was that developed by Barman & Geber (Progress in Brain Research, 81:117-129, 1991), in which the RDA unitary action potentials serve as triggers for averaging 1 sec segments of the sympathetic discharge envelope wave. The activities of 17 cells yielded sympathetic slow wave averages of 2-3 Hz (synchronous with the heart rate), while the activities of 38 cells failed to correlate with the sympathetic discharge. Baroreceptor reflex activity (induced with IV phenylephrine or balloon-inflation in the aorta) revealed that, of these 17 cells, 8 were sympathoexcitatory, 5 were sympathoinhibitory, and 4 that RDA activity was heterogeneous. This indicates that the RDA is a heterogeneous population of cells, with cardiac-related sympathoinhibitory neuronal activity not as predominante as our previous methods suggested.


Sympathetic nerve discharges have both fast sympathetic-specific rhythms (2-6 Hz and 10 Hz) and respiratory rhythm. In 13 micodilator-decarcerated, paralyzed cats, we recorded phrenic and cervical sympathetic (CS) nerve discharges together with discharges of neurons in ventral medullary regions (Cooper et al., 1989). Of 208 recorded units, 26 had distinct and significant CS coherence peaks (range 2.1-17.8 Hz). Of these, 12 had peak coherence values > 0.1 (range 0.11-0.71, mean 0.280±0.23SD). The other 14 units had peak coherence values < 0.1 (range 0.05-0.09, mean 0.070±0.01SD). Most of the neurons (21/26) had a respiratory modulated rhythm (usually tonic I or E modulated) as determined by the eta-square test (Orem & Dicke, J. Neurophysiol. 50:1099-1107, 1983). These results suggest that the combination of fast and slow (respiratory) rhythms seen in CS discharges originate in the medulla. (Supported by N.I.H. Grant HL-73500.)

MEDIUM-SIZED RETINAL GANGLION CELLS PROJECTING TO THE GROUND COLLIUOUS SUPERIOR IN THE RABBIT, R.E. BLANCO AND N. LUSO. Inst. of Neurobiology and Dept. of Anatomy, U.P.R.C.M., San Juan, P.R. 00901.  

In the thirteen-lined ground squirrel (Spermophilus tridecemlineatus), retinal ganglion cells have been classified into 3 groups on the basis of their soma cell diameter: large (>14 μm), medium (10-14 μm) and small cells (6-10 μm; Flores, 1983). Of these, mostly small and medium-sized cells project to the superior colliculus (SC) and mostly large cells project to the dorsal lateral geniculate nucleus (Lugo-Garcia and Klicier, 1988). In order to characterize further the ganglion cells projecting to the SC, a retrograde tracer, cholera toxin subunit B (CTB), was injected into the superficial layers of the SC. After a survival period of 5-7 days, the animal was perfused, the retina dissected and the CTB immunohistochemically detected with a goat anti-CTB antiserum (List Biologicals, 1:2,500). Labeled cells were drawn and measured with a Neurohalla and an ocular. Our results confirm that retinal ganglion cells projecting to the SC are medium and small-sized. Most medium-sized cells had diameters of 10-11 μm. Their dendritic trees were extensively labeled. They differed in soma shape, number of primary dendrites, density of branching, symmetry of branching and pattern of dendritic ramification within the inner plexiform layer. This morphological diversity suggests that medium-sized cells are composed of more than one population. (Supported by ONR grant N00014-89-J-3070 & NIMH Grant MH-48190.)


Spectral analysis of heart rate (HR) and arterial pressure (AP) variables in man as well as in experimental animals displays two major components: a low frequency (LF; 0.04-0.15Hz) and a high frequency respiratory related (HF; 0.25 Hz) rhythm. HF is considered a marker of vagal modulation while LF is considered a behaviorally unmodulated component of HR fluctuation. In a previous study (Montano et al., 1980; 40:21-32, 1992) spectral analysis of cardiac sympathetic effenter discharge, in the range between 0 and 0.5 Hz, showed a predominance of LF oscillation, highly correlated with the LF detectable in HR and AP. This study was undertaken to evaluate whether LF rhythmicity could also be present in the variability of discharge of single medullary neurons involved in cardiovascular regulation. We analyzed, by means of autoregressive analysis algorithms, AP and impulse activity of single units recorded from lateral tegmental field, medullary raphe and rostral ventrolateral medulla in decerebrate and in urethane anesthetized, baroreceptor-denervated cats. The activity of medullary neurons was correlated to higher frequency components (2- to 6 Hz or 0.5 Hz) in sympathetic nerve discharge. Power spectra of single neurons discharge displayed an LF oscillation, highly coherent (between 0.6 and 0.8Hz) with the LF detectable in systolic AP. In conclusion we described for the first time that the impulse activity of single medullary neurons contains an LF rhythmity similar to that detectable in spectral analysis of the cardiovascular variables, thus raising the possibility of an involvement of these nuclei in generating this oscillation.
485.3 NON-SPECIFIC EFFECTS OF ANTIANGIOTENSIN II AND ITS AGONISTS AND ANTAGONISTS IN THE SUPERFICIAL LAYERS OF THE RAT SUPERIOR COLICULARIS. J. C. Mandel, R. L. Mandel, S. C. Mandel, Dept. of Ophthalmology, Physiology and Biophysics, Neurosurgery; Faculty of Medicine, Universidad de Sheffield, Sheffield, UK.

We have reported previously (Mandel et al., ARVO'94) that activation of angiotensin type 1 (Ang I) receptors in the superficial layers of the rat superior colliculus (SC) had a significant effect on evoked potentials (VEP). Some of our initial observations have suggested that the ‘early’ and ‘late’ components of the VEP complex are ‘on’ and ‘off’ responses respectively, and were differentially affected by the peptide. In the attempt to further characterize these effects, we investigated the impact of various concentrations of Ang I and specific agonists and antagonists (DUP 753, CGP 42112A). We also studied the effects of Ang I on separate ‘on’ and ‘off’ response components. Experiments were carried out on unanesthetized adult rats (15-16 weeks). A recording-injecting microelectrode filled with the peptide was implanted in the superficial layers of the SC in order to record VEP. Visual stimulation was provided by either a diffuse flash or a small testing spot (diameter of 500μm) placed in the corresponding field to evince ‘on’ or ‘off’ responses. The substance was injected at various concentrations (from 10-5 M to 10-2 M). Inhibitory effects of Ang I were observed at concentrations greater than 10-6 M and we were able to observe a differential effect on ‘on’ and ‘off’ responses. Increasing concentrations of Ang I gave different effects depending on its concentration. At lower concentrations (10-6 M), there were no significant changes in the amplitude of the evoked potentials. However, at 10-5 M, a concentration known to activate AT receptors predominantly, there was a reduction in the amplitude of the potential (5%) similar to that observed with Ang I. Injection of the AT antagonist DUP 753 together with Ang I yielded no significant changes in the amplitude of the responses. Further, in our original observation, Ang I equally reduced the amplitude of both the ‘on’ and ‘off’ potentials suggesting a non-specific effect for the peptide. These results contribute to earlier findings that Ang I has a suppressive effect on the superficial collicular layers in the adult rat. These responses appear to be mediated by the sole activation of AT receptors and tend to be nonspecific. Supported by MRC of Canada and Ciba-Geigy Ltd.


Previous work from our laboratory suggested that multiple adrenergic receptors were present in the superficial layers of the hamster’s superior colliculus (SC), and that the substantial number of α2 and β2 receptors may be located presynaptically on retinal and/or corticostriate mesotelencephalic terminals. The functions of these receptors were tested by intracellular or whole-cell recording in a perfused SC slice that included only the optical nerve, substantia nigra, and mesencephalic stimulation of retinotectal fibers. Addition of norepinephrine or epinephrine to the bath or application of microperfusion hyperpolarized ~55% and depolarized ~35% of 207 SC neurones tested, respectively, enhanced the membrane potential by ~3 mV. On average the membrane potential hyperpolarized from ~70 ± 1.5 mV to ~74.3 ± 0.9 mV when voltage clamped at ~70. The results indicate that the hyperpolarized neurones had net outward currents of 18 to 35 pA and depolarized cells had inward currents of 26 to 38 pA. The EPSP was reduced from 10.8 ± 3.7 mV to 6.7 ± 3.7 mV by NE, but the decrease was not correlated with any change in membrane potential. To date, tests with more selective ligands have shown that (S)-3-methyl-α-aminonitrone (α2), dobutamine (β1), and isoproterenol (β2 and β3) have effects on SC cells similar to those of NE. In conclusion, our data indicate that the adrenergic neurones, which could be located presynaptically on retinal or corticostriate mesotelencephalic terminals, produce significant effects on the membrane potentials of SC neurones. Supported by NIH NS 9342-90 and NS 32540.

485.5 EFFECTS OF ADRENERGIC AGENTS ON NEURONAL RESPONSES IN THE HAMSTER’S SUPERIOR COLICULARIS. Y. Zhang, R. W. Rhodes, and R. J. Monnery. Dept. of Anatomy, Medical College of Min. Toledo, OH 43699.

Radioimmunoassay binding experiments have demonstrated the presence of multiple adrenergic receptor subtypes in the hamster’s superior colliculus (SC) and the present experiments were undertaken to characterize the pharmacology of adrenergic effects on the visual, optic chiasm (OX), and visual cortical (VCTX) responses of PNs in the hamster. Of 40 neurones tested visually, 90% were suppressed by NE to 39.4 ± 28.4% of control responses and 10% were excited. Similar results were obtained during OX stimulation (N=28 cells). Both α1 agonists (phenolamine) and β agonists (dopamine) had marked effects on SC neurones similar to those of NE. Dobutamine (N=16 cells) had effects that varied, while methoxamine suppressed 4 cells and excited 6. The effects of NE were strongly antagonized by α1 antagonists and β adrenergic antagonists (P  and P2). α2 agonists were ineffective in nearly all neurones. These results indicated that α2 agonists completely blocked NE-evoked excitation, but did not reduce the suppressive effects of this amin. Thus, the suppressive effects of NE upon SC neurones appear to be mediated primarily by α1 and β2 receptors while excitation involves α1 receptors. No α2 adrenergic receptor subtype was specifically associated with either the retinotectal or corticostriatal pathway and experiments carried out to date have not allowed us to determine whether any of the effects of NE are exclusively pre- or post-synaptic. Supported by NIH NS 9208211 and NS 32540.


Neurones in the superficial grey layer (SGS) of the SC are innervated by the retina and visual cortex. SGS has 3 sublayers SGS1, SGS2, Retinal inputs dominate in SGS, SGS3, and SGS4 (from the surface) cortical inputs are prevalent. The NMDA antagonist AP5 reduces visual responses in 60% of SGS neurones indicating that NMDA receptors participate in these responses. Many of these cells are in SGS4. To test whether the contribution of NMDA receptors to visual responses is derived from cortical input to SGS, multibarrelled pipettes were used for single unit extracellular recording and iontophoresis of AP5, NMDA, AMPA and NMDA/AMPA in cats anaesthetised with halothane/N₂O/O₂ and paralysed with gallamine. The effects of NMDA receptor selective applications of AP5 on visual responses were studied before and during inactivation of the visual cortex with topical lignocaine. In 8 neurones where AP5 reduced the response to visual stimulation, the visual response was resistant to the effects of AP5 following cortical inactivation. Since the cortex is considered to influence the directional bias of SGS neurones, the effects of AP5 (n=17) and cortical inactivation (n=16) on directional responses were compared. Neither procedure altered the directional bias of SGS neurones. These data imply that, considerable NMDA receptor mediated input to SGS is mediated via the cortex. However, the cortex may not influence the directional bias of SGS neurones.

(Supported by the MRC.)


485.7 GAMMA-AMINOBUTYRIC ACID (GABA) IS EXPRESSED EARLY IN PREMATURAL DEVELOPMENT IN THE CAT SUPERIOR COLICULARIS. J.P. Henri*, M.T. Simon, F.T. Banfo, and R. R. Mige*. Dept. of Anatomy and Neuroscience Ctr., Louisiana State Univ. Med. Ctr., New Orleans, LA 70112. GABA is expressed prenatally in the superior colliculus (SC) in the cat adult, but the time of expression of GABA in this structure is not known. We therefore studied the expression of GABA-ir neurones in kittens ranging from 2 days to postnatal day 60 using antibody immunocytochemistry. Labeled cell position and density were determined using a neuron tracing system. At E24, GABA-immunoreactive neurones were observed in the SC and were concentrated deep within the superficial layers of SC than deeper. Some cells in the deeper layers were also present on the superolateral surface of the SC, reminiscent of cells in the process of migration. The number of GABA-ir cells increased roughly eight fold from E30-E46, and the adult pattern of cell distribution was clearly evident during this period. There was a two-fold decrease in GABA-ir cell number between E46 and P60. Cells also increased in maturity during this period. Based upon labeling of adjacent sections with antibody to vimentin, a glial fibrillary acidic protein (GFAP), we believe that most of the GABA labeling is in neurons and not glia throughout this developmental period. In summary, GABA is expressed very early in prematurnal development in the SC, which is consistent with the endogenous binding protein calcium in this structure. GABA is expressed in some cells before they migrate into SC and clearly precedes the formation of the adult pattern of GABA neurones. The increase in number of GABA-ir cells may be due to loss of expression of the neurotransmitter or to cell death. Supported by NIH EY02973.


Parvalbumin (PV) is found in neurones in some neurones in the cat superior colliculus (SC) are projection neurones that form a dense sublamina tier in the deep superficial grey and upper laminar regions of the SC. Calbindin (CB) neurones are multiform neurones that form a sublamina tier with the PV neurones in the superficial grey and upper laminar regions of the SC. We have examined kittens aged E24 to P60 to determine when these tiers develop. Sections were immunocytochemically treated with monoclonal antibodies directed against PV and CB. Labelled neurones were then scattered throughout the SC as well as within the subventricular zone. By E28-30, GABA-ir cells and neurones were more densely concentrated within the superficial layers of SC than deeper. Some cells in the deeper layers were also present on the superolateral surface of the SC, reminiscent of cells in the process of migration. The number of GABA-ir cells increased roughly eight fold from E30-E46, and the adult pattern of cell distribution was clearly evident during this period. There was a two-fold decrease in GABA-ir cell number between E46 and P60. Cells also increased in maturity during this period. Based upon labeling of adjacent sections with antibody to vimentin, a glial fibrillary acidic protein (GFAP), we believe that most of the GABA labeling is in neurons and not glia throughout this developmental period. In summary, GABA is expressed very early in prematurnal development in the SC, which is consistent with the endogenous binding protein calcium in this structure. GABA is expressed in some cells before they migrate into SC and clearly precedes the formation of the adult pattern of GABA neurones. The increase in number of GABA-ir cells may be due to loss of expression of the neurotransmitter or to cell death. Supported by NIH EY02973.
CALCUT BINDING PROTEIN CALBINDIN D-28K IN THE PRETENTIC
AND ACCESSORY OPTIC SYSTEM OF THE RABBIT. B. Nune Cordero,
J.J. van der Want, G. Butler and R.B. Mize. SPON: European Neuroscience
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The calcium-binding protein calbindin-D-28K (CIB) is a highly selective marker
of specific cell types in the CNS. In the cat pretectum (PT), CB cells form four
cell clusters, two of which lie partially within the nucleus of the optic tract (NOT).
CB cells are mostly large projection neurons that precisely overlap the retinal input
to PT and cross nuclear borders. In this study, we determined whether these same
cell clusters are present in the rabbit, a lateral-eyed species with a well developed PT.
CB cells through the NOT and accessory optic system (AOS) nuclei of three
rabbits were incubated with an antibody directed against CB and visualized using
silver-gold enhanced DAB. A few neurons in the retinal NOT were CB positive,
while the caudal NOT was devoid of CB neurons. Unlike the cat, separate dense
clusters of neurons were not observed in the PT of rabbit. However, clusters of
CB neurons were observed in the caudal portion of the dorsal terminal nucleus
(DTN) and in the transition area of the DTN and interstitial nucleus of the superior
fasciculus posterior fibers (mSTP). However, the medial terminal nucleus (MTN)
was completely free of CB neurons. In contrast to cat, the clusters of CB neurons
in the AOS fell within the established borders of these nuclei. In addition, there
was no precise overlap between CB cells and retinal afferents in the rabbit PT and
AOS. CB appears to label different cell classes in the two species. Supported by
NIH EY02973.

CROSS-MODAL INHIBITION IN MULTISENSORY NEURONS IS
BASED ON UNIMODAL RECEPTIVE FIELD ORGANIZATION. D.C.
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A fundamental principle of multisensory integration is that spatially
 coincident stimuli from different modalities enhance one another’s
effectiveness, whereas spatially disparate stimuli may depress one’s
effectiveness. The present experiments in cat superior colliculus (SC)
were designed to examine the basis for this cross-modal inhibition,
and to determine whether the excitatory-inhibitory organization of
multisensory RFs is significantly different from that of unimodal RFs. A
total of 114 (55 unimodal; 59 multisensory) neurons were examined.
Surprisingly, each multisensory RF was organized in fundamentally the
same manner as its unimodal counterpart. However, the incidence of
inhibitory regions borders differed across modalities. The majority (81%)
of auditory RFs had inhibitory regions, but far fewer visual (22%) and
somatosensory (15%) RFs did. In many multisensory neurons RF inhibitory
borders were not modality-specific (e.g., auditory stimuli would suppress
visual excitation); in others, inhibitory regions were modality-specific
and this was most common for visual inputs (e.g., visual inhibits only
visual). These data indicate that while cross-modal inhibition in SC is
due to the presence of nonspecific inhibitory regions, inhibitory regions
themselves do not guarantee such effects. In these cases the inhibitory
effect is on the input rather than on the target multisensory SC neuron.
Supported by NIH grant NS2543.

SIMULTANEOUS RECORDINGS OF MULTI-SINGLE NEURAL ENSEMBLES IN THE SUBCORTICAL VISUAL SYSTEM REVEAL PRESENTATION OF OSCILLATORY WAVES. Rowshanak Hashemiyoon1 and John K. Chapin. Department of Physiology & Biophysics, Hahnemann University, Philadelphia, PA 19104

We have spontaneous oscillations (10-40Hz) that are ubiquitous in the subcortical retino-geniculo-striate visual system. These oscillations are broadcast from the retina to synchronize unit activity across multiple visual pathways. These are simultaneously recorded upon recording of multi-single units in the visual midbrain, thalamus and optic tract. Further, we investigate the spatiotemporal structure of these oscillations, electrode arrays consisting of 16-microelectrodes (18 0.5 mm) were chronically implanted across the stratum griseum superficiale (SGS) of the superior colliculus. Under anesthetized and awake conditions spatiotemporal synchronous discharge rhythms recorded from these electrodes exhibited variable phase angles of up to 15ms, indicating propagation of activity waves across the SGS. A small (14") flashing light (1s ON, 1s OFF) in the contralateral visual field suppressed the oscillatory activity of the SGS. LIGHT-OFF produced a phase resetting oscillatory rebound, without latency differences by up to 15ms, suggesting propagation of waves away from the LIGHT-OFF point. Other studies investigated the effect of non-photopic, non-patterned stimulus. Whereas non-pat-
tempered flashing circles of light produced simple LIGHT-OFF suppression, addition of a dark bar to the middle of the circle produced a more complex response, consisting of an additional oscillatory peak during the LIGHT-OFF period. In conclusion, the propagating spatiotemporal pattern of these oscillations may not only integrate sensory processing across the subcortical visual system, but might also carry a distributed code for visual sensory information. Supported by NS25722 to JKC.

GABAa MEDIATED DISINHIBITION OF THE SUPERIOR COLICULUS RESTORES VISUAL ORIENTING BEHAVIOR IN RABBITS IN THE PREVIOUSLY HEMIANOPTIC VISUAL FIELD OF THE CORTICALLY BLIND CAT. V. Ciaramarco1, J.S. Durmer, W.E. Todd and A.C. Rosenquist
Dept. of Neuroscience, Univ. of Penn, Phila., PA 19104

Following unilateral removal of all known visual cortical areas, a cat is rendered hemianopic in the contralateral visual field as measured by visual perimetry and other behavioral tests. It has been shown that visual orientation behavior can be restored to the previously blind hemifield by transection of the commissure between the two colliculi, or by destruction of the contralateral substantia nigra. (Sprague, I.M., Science 151:1544-1547, 1966; Wallace, S. F. et al., J. Comp. Neurol. 296:222-252, 1990) It was hypothesized that the mechanism mediating recovery is disinhibition of the SC ipsilateral to a cortical lesion.

We directly tested this hypothesis by reversibly disinhibiting the SC ipsilateral to a cortical lesion with bicuculline, a GABAa antagonist. In accordance with the model, disinhibition of the SC ipsilateral to the cortical lesion restored visual orienting responses in the hemianopic visual field for a period of several hours. Control injections of saline had no effect on visual orienting behavior. Conversely, inhibition of the SC with muscimol, a GABAa agonist in a normal animal renders the animal unable to detect and orient to visual stimuli in the hemisphere contralateral to the SC.

(Supported by EY02654, 2 T32 EY07035 and 2 P01 EY01583.)
485.15 RECOVERY OF VISUAL ORIENTING IN THE HEMIANOPIC CAT AFTER SMALL SUBSTANTIAGeba LIGATION IS NOT ABOLISHED BY SUBSEQUENT ENLARGEMENT OF THE INITIAL LESION

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Unilateral, 90% of all known visual cortical areas in the cat renders the animal hemianopic in the contralateral visual field as measured by visual perimetry and other behavioral tests. Visual orientation behavior is retained in the lesioned eye by an activation of the unlesioned sensory system as a result of increased visual input to the unlesioned visual cortex. Performance of the visual perimetry tests were normal in both eyes. After unilateralling the unlesioned eye, normal visual orientation behavior was retained, but pretend to normal visual orientation behavior is not abolished by subsequent enlargement of the initial lesion.

Supported by EY02654 and 2 T32 EY07015.

485.16 IOTIOTIC ACID LESIONS OF THE PEDUNCULOPONTINE NUCLEUS RESTORE VISUAL ORIENTING RESPONSES IN THE PREVIOUSLY HEMIANOPIC VISUAL FIELD OF THE CORRECTLY BLIND CAT

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The unilateral removal of normal visual cortical areas in a cat results in an enduring hemianopia as measured by visual perimetry and other behavioral tests. This unilateralling the properly visual cortex with ibotenic acid destruction of a critical zone in the substantia nigra pars reticulata (SNpR) contralateral to the cortical lesion (Wallace, S.F. et al., J. Comp. Neurol. 296:222-259, 1990). Paradoxically, large lesions to the SNpR which include but extend beyond this critical zone fail to restore visual orienting behavior.

In an attempt to understand this paradoxical finding, we made unilateral visual cortical lesions followed by ibotenic acid lesions of the contralateral SNpR critical zone in 5 cats. All cats recovered visual orienting behavior in the hemianopic field. In these recovered animals, the original SNpR critical zone lesion was subsequently enlarged with ibotenic acid an average of 3.4 weeks after the initial lesion. None of the 5 animals showed a loss of recovery. This finding argues strongly for the role of plasticity in nigrostriatal circuitry and visual orienting behavior. The time between a critical zone lesion and an enlargement of this lesion appears to be sufficient for compensatory mechanisms to maintain recovery.

(Supported by EY02654 and 2 T32 EY07015)

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Most afferents to the cat superior colliculus (SC) are present at birth, including those from the substantia nigra. It is not known when these reach the SC during prenatal development. We have therefore studied the development of innervation of the SC by SN fibers in fetal tissue using the cytochrome d oxygen Dil. Dil crystals were placed within the SN in blocks of brain perfused at ages E46, 46, 51, 57, 57, and 59 days post-conception. These blocks were stored in fixative in an oven at 37°C for 4-5 days, then sectioned and examined with an epifluorescent microscope. Labeled fibers were traced with a neuron tracing system. No Dil fibers were present in the superior colliculus at birth, although they were present in the oculomotor nucleus. By E46, Dil fibers were present in the deep layers of the caudal SC, but were not present in the superficial layers. The fibers were thin and had few varicosities. Myelinated fibers were seen on or near the optic tract. Few fibers were found in the rostral SC. The labeled fibers were confined primarily to the unilateral SN. By E51, the number of labeled fibers in the SC had increased. By E57, large labeled fibers were seen on the intermediate gray layer (IL) of the caudal SC. The fibers at this age were thicker and some had numerous varicosities. Fiber density increased at E57 and P1, but no fibers were found above the IL at even P1. Thus, a sharp border between the superficial and deep layers is maintained at this age. No obvious fiber patches were observed at any age. The patch-like organization of SN fibers seen in the adult must therefore develop postnatally. SN fibers reach the SC about 2 weeks after retinal afferents have innervated SC, and by SC lamination is complete. Supported by NIH EY-02973.

485.18 EVIDENCE THAT AREA 17 CORTICAL SYNAPSES IN THE CAT SUPERIOR COLICULARIS ARE GLUTAMATERGIC. G.D. Butler, R.H. Whitworth* and R.B. Mize.
Dept. of Anatomy and the Neuroscience Center, Louisiana State Univ. Med. Ctr., New Orleans, LA 70112

We have previously shown that antibodies to the excitatory amino acid neurotransmitter, glutamate, label at least two types of synaptic terminals in the cat superior colliculus (SC). One type contains large round synaptic vesicles and appears to stain the nigrostriatal synapses. The second type contains smaller round vesicles and dark mitochondria (RSD). The origin of the latter type is unknown. To determine if RSD arise from synaptic vesicles, we made unilateral aspiration lesions of area 17 in three cats and waited 5, 14, and 28 days before sacrifice. We then examined the distribution of glutamate in the SC of these cats using electron microscopy post-embedding immunocytochemistry and colloidal gold-conjugated antibodies. On the side contralateral to the lesion, both RT and RSD axon terminals were labeled by anti-gluatamate, as in the normal SC. Some conventional dendrites and myelinated axons were also labeled. On the lesioned side, morphologically normal RSD terminals were infrequently observed. However, synaptic terminals in the initial stages of electron dense degeneration that had a dark ground and irregularly shaped swollen vesicles were labeled by anti-glutamate. Axon terminals and myelinated axons undergoing neurofilamentous hypertrophic degeneration also were often labeled. By contrast, terminals in advanced stages of degeneration were not synaptically active and contained little or no glutamate. These results confirm that both major sets of afferents to the cat SC contain glutamate; RT terminals from the retina and RSD terminals from visual cortex. It is possible, however, that not all terminals from these sources contain glutamate because a few RT and RSD terminals were not labeled by the antibody. Supported by NIH EY-02973.

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486.1 CANNABINOID AGONISTS ATTENUATE DOPAMINE-MEDIATED ROTATION IN RATS WITH UNILATERAL 6-HYDROXYPAMINOQUINOLINE LESIONS. L.A. Anderson*, J. Anderson, J.R. Walters and T.N. Chase, ETH, NINDS, NIH, Bethesda, MD, 20892, USA

The presence of cannabinoid receptor (CB-1) and D2 dopamine receptor antagonists and/or cannabinoids in the substantia nigra, caudate nucleus, and striatum, suggests that cannabinoids mediate effects on the striatum and striatal pallidum. This study was designed to examine this hypothesis. Basal ganglia (striatum, caudate nucleus, and putamen) were lesioned with 6-OHDA or monoamine oxidase-B (MAO-B) and subsequent rotation of these animals was measured. Bilaterally lesioned rats were administered D1- and D2-selective cannabinoid agonists or antagonists in the striatum and glutamatergic systems. Rats were allowed to habituate to the rotarod for 2 days before the baseline rotation was measured. Animals were sacrificed at 2, 5, 10, 15, 20, and 25 mg/kg i.p. with the cannabinoid receptor agonists. 15 mg/kg i.p. was the lowest dose that produced a reduction in the baseline rotation. The administration of 15 mg/kg i.p. reduced the baseline rotation by 50% and 25 mg/kg i.p. reduced the baseline rotation by 75%. The administration of 30 mg/kg i.p. reduced the baseline rotation by 90% and 40 mg/kg i.p. reduced the baseline rotation by 100%. This study demonstrates that cannabinoids can attenuate the baseline rotation of 6-OHDA lesioned rats and that the cannabinoids used in this study are effective in reducing the baseline rotation of 6-OHDA lesioned rats.

486.2 EFFECT OF SUBSTANCE P (NK1) RECEPTOR BLOCKADE ON CATALYSIS INDUCED BY DOPAMINE AGONISTS. Jeff Anderson* and Thomas N. Chase, Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892

Systemic administration of D1 or D2 receptor antagonists induces catalepsy, a behavior defined as the long-term maintenance of an abnormal posture. Catalepsy is often considered an animal model of neuroleptic-induced parkinsonism. Subsequent to a retinal output pathway lesion, the lateral geniculate body is affected in striatal lesions. Bilaterally lesioned rats were treated with the non-peptide NK1 antagonist CP 99,994 (0.5, 2.5, or 10 mg/kg i.c.v.) or saline 15 min after administration of either the D1 agonist SCH 23390 (0.5 mg/kg i.c.v.) or the D2 agonist raclopride (2.5 mg/kg i.p.). Catalepsy was evaluated by the vertical grid test every 20 min for 160 min. CP 99,994 (2.5 and 10 mg/kg) decreased the catalepsy produced by raclopride by 46% and 78%, respectively. In contrast, CP 99,994 did not diminish the catalepsy induced by SCH 23390. These findings suggest that blockade of NK1 receptors reduces catalepsy produced by D2 but not D1 antagonists and that NK1 antagonists may be important in modulating behavioral responses mediated by D2 receptors. In addition, NK1 antagonists may have therapeutic potential in alleviating motor side effects associated with neuroleptic treatment.
Concurrent stimulation of D1 and D2 receptors by systemically administered dopamine agonists inhibits the activity of substantia nigra pars reticulata (SNr) neurons in rats with 6-hydroxydopamine (6-OHDA)-induced nigrostriatal cell loss. In order to investigate possible sites of action, the D2 agonist SKF 38393 was locally administered into the striatum (0.15 nmol) or the substantia nigra (9-17 nmol) of gallamine-paralyzed rats with unilateral 6-OHDA lesions (6-9 wk post-lesion) while the activity of individual SNr neurons was recorded extracellularly. Rats were tested for apomorphine (0.05 mg/kg, s.c.)-induced contralateral rotation 1-2 wk prior to study. An infusion of SKF 38393 in the striatum (n=7) or in the substantia nigra (n=7) produced no significant changes in the firing rate of SNr neurons. The D2 agonist quinpirole (0.3 mg/kg, i.v.) increased firing of SNr neurons (mean±SE: control 0.02±0.06; quinpirole 0.03±0.02; n=7). However, quinpirole administered after stratal infusion of SKF 38393 (n=7) or after nigral infusion of vehicle (n=6) produced no net change in the firing rate of SNr neurons. These findings suggest that the response to intravenous quinpirole depends on whether D2 receptors are stimulated by SKF 38393 in either the striatum or the substantia nigra. However, the stimulation of only D2 receptors by SKF 38393 at either striatal or nigral sites is not sufficient to alter the activity of SNr neurons. Explanations include the possibility that D2 receptors at another site or at a combination of sites including striatum and substantia nigra are required to produce changes in the activity of SNr neurons.

The mitochrondrial toxin 3-nitropropionic acid induced oral movements in rats. O.A. Andersen* and H.A. Jørgensen†. Deps. of Physiology and Psychiatry. Univ. of Bergen, N-5009 Bergen, Norway.

Tardive dyskinesia (TD) is a serious side effect of long-term neuroleptic treatment. The mechanism of TD development is still unknown. It has been proposed that TD may be a result of neuroleptic-induced excitotoxic neuronal damage in the striatum. To investigate the excitotoxic effects of 3-nitropropionic acid (3-NP), an inhibitor of energy metabolism which has been shown to induce striatal lesions due to glutamate receptor stimulation (in situ excitotoxicity), was studied in a rat model of TD. Three groups of rats (female Sprague-Dawley rats, n = 14-15/group) were treated continuously for 1 month with 3 doses of 3-NP (4, 8 and 12 mg/kg/day) administered SC with osmotic minipumps. Empty plastic tubes were inoperated SC to control rats. The behavior was videotaped regularly and vacuous chewing movements (VCM), a putative sign of TD, were monitored for 6 months, as well as the total behavior, were recorded.

3-NP induced a dose-dependent increase in VCM in the period from 2 to 4 weeks. The effects were somewhat different among rats; while the highest dose of 3-NP had significantly more VCM than controls and the rats receiving the lowest dose were at control level, 3-NP also dose-dependently decreased the general motor activity.

The effect of 3-NP on VCM suggests that striatal glutamate receptor stimulation may underlie the development of VCM in rats and maybe TD in humans.

Dopamine receptor agonists and forskolin. Similarly potentiate responses evoked by excitatory amino acid receptor agonists in rat neostriatal neurons. M. Day, C. Cobelli, N.A. Buchwald, and M.S. Levinger. National Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

Our laboratory has studied the physiological interactions between dopamine (DA) and excitatory amino acids (EAA) in the neostriatum. We have shown that DA potentiates the evoked responses elicited by N-methyl-D-aspartate (NMDA) receptors but attenuates responses mediated by glutamate or activation of non-NMDA receptors. The present experiment was designed to examine the possibility that DA's ability to activate receptors on NMDA receptors involves alterations in the adenyl cyclase transduction cascade. Intracellular recordings were made from neostriatal neurons in brain slices using standard techniques. Similar to previous findings, iophotonic or bath application of the DA D1 receptor agonist SKF 38393 potentiated NMDA-evoked responses by increasing the maximum amplitude, half amplitude duration and number of action potentials evoked. SKF 38393 also enhanced glutamate-evoked responses, but to a lesser degree. Receptor antagonists were used to evaluate the specificity of the agonists' actions. Bath application of forskolin, an activator of the adenyl cyclase which increases cAMP, mimicked the effects of D1 DA receptor activation and enhanced responses evoked by iophotonic application of NMDA. Forskolin also enhanced DA responses and the existence of such potentiation is consistent with a model in which DA, acting through activation of D1 receptors, enhances cAMP-evoked responses through a cAMP-dependent mechanism. Supported by USPHS HD 00958.

AP-1 DNA binding activity in striatum: dopaminergic regulation. K.-X. Huang* and J.R. Walters. ETR, NINDS, NIH, Bethesda, MD 20892.

Stimulation of dopamine (DA) receptors in the basal ganglia affects expression of c-fos, while interruption of c-fos expression in the striatum with antisense DNA reduces behavioral response to DA agonists1,2. Since Fox and Jun proteins influence cellular function by forming AP-1 which binds to DNA sites regulating gene transcription, detection of DA receptor activation by stimulation on striatal AP-1 DNA binding activity were examined using a gel shift assay. Nuclear protein extract (10 μg), prepared from homogenized striatum, was incubated with the striatal DNA containing 42 bp of sequence AP-1 or CREB consensus sequence. In normal rat striatum there was a high level of AP-1 binding. i.p. administration of (+) SKF 38393 (5 mg/kg, n=9) or quinpirole (0.25 mg/kg, n=8) significantly affected AP-1 binding 2 hr later, while did not a combination of these drugs (n=7). In striata of rats treated with reserpine (10 mg/kg, s.c.) for 4-7 hr, AP-1 binding was increased by 90% (n=8, p<0.01). AP-1 binding was also increased by 80% (n=7, p<0.01) 12 weeks after 6-OHDA-induced lesion of the nigrostriatal pathway and by 45% (n=5, p<0.05) 8-9 months later. In 6-OHDA-lesioned rats, a combination of (+) SKF 38393 (10 mg/kg) and quinpirole (0.25 mg/kg) further increased AP-1 binding by 76% (n=6, p<0.01). Striatal CREB DNA binding activity was not significantly affected in reserpine or lesioned rats. Thus, changes in Fox synthesis could be involved in DA agonist behavioral effects.2,12 are not reflected in total striatal AP-1 binding 2 hr after agonist treatment in normal rats, but are evident in 6-OHDA-lesioned rats. The observation that striatal AP-1 DNA binding activity is enhanced by DA depletion as well as by DA receptor stimulation after DA cell destruction suggests that different underlying mechanisms are involved. 'Sommer et al., Neuroreport 5: 277, 1993; 'Draganow et al., Neuroreport 5: 305, 1993.

Regulation of neostriatal neurons by cAMP-dependent mechanisms. C.S. Coletti and M.S. Levine. Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

The purpose of the present study was to examine the possibility that cAMP-dependent regulation of synaptic transmission occurs in the neostriatum. Intracellular recordings were obtained from neostriatal neurons in slices using standard techniques. Depolarizing and synaptic potentials (DPSs) were elicited by local electrical stimulation. Bath application of forskolin (FKK), an activator of adenylyl cyclase, enhanced DPS amplitude (155±8% (mean±se) of control) and duration (122±6% of control). This potentiation of DPS was due to an increase in the frequency of invasion of the GABA receptor antagonist bicuculline, and was not seen with the inactive FKK analog 1,3-didehydroforskolin. Rp-Camps, an inhibitor of cAMPT-dependent protein kinase (PKA) was applied in additional experiments. Rp-Camp (100μM) significantly attenuated FKK's enhancement of DPS amplitude. Moreover, the PKA activator Sp-Camps (100μM, 10 min) also enhanced DPS amplitude. These results suggest that FKK is acting through a PKA-dependent mechanism to enhance DPS amplitude. Interestingly, in some neurons, treatment with Rp-Camps clearly depressed the evoked response. This inhibition was also seen when the intracellularly-activated inhibitor PKI was applied via the recording electrode. In contrast, bath application of the phosphatase inhibitor, okadaic acid (10μM), increased DPS amplitude. These results indicate that, at least under these experimental conditions, activation of PKA and phosphatase activity regulates DPS amplitude. Taken together, these results suggest a role for the adenylyl cyclase cascade in the regulation of synaptic transmission in the neostriatum. Supported by USPHS HD 00958.
DIZOCILPINE MALEATE DIFFERENTIALLY INFLUENCE THE POSTSYNAPTIC EFFECTS OF NIGROSTRIAL DOPAMINE DEAFFERENTATION AMONG SUBPOPULATIONS OF STRIATAL NEURONS.

P. Sain* M.D. Hajii, A. Nicoullon and L. Kerkerian-Le Geoff, Cellular and Functional Neurobiology Laboratory, CNRS, 13402 Marseille cx 20 (France).

It was previously reported that following dopamine (DA) D2 receptor blockade or DA-depletion, neurtensin (NT) immunoreactivity (IR) is elicited in two distinct subpopulations of rat striatal neurons. Colocalized Fos-IR, interpreted as an indicator of enhanced neuronal activity, was observed in one of these subpopulations following D2 blockade (Neurosci. 57: 649-60, 1993). In this study, the degree of colocalization of Fos- and NT-IR in striatal neurons in response to acute midbrain 6-hydroxydopamine (6-OHDA) lesions was studied. It was observed that a subpopulation of smaller neurons exhibited light NT-IR and frequent colocalization with Fos-IR was predominant following reperfusion administration, mainly in the dorsolateral quadrant of the striatum. Another subpopulation of larger, intensely NT-immunoreactive neurons that was rarely colocalized with Fos-IR was observed following all the drug treatments, but was present almost to the exclusion of the smaller cell type three days following 6-OHDA lesions, mainly in the dorsomedial and ventrolateral portions of the striatum. The present data are consistent with two subpopulations of striatal neurons that accumulate NT following DA depletion. The possibility is considered that one subpopulation accumulates NT in response to coordinate increases in neuronal activity and NT synthesis, and the other as a result of decreased release. Support: NIH NS-23805, T32 NS-07254, and the United Parkinson Foundation.

BASAL GANGLIA AND THALAMUS IX

486.10


The accumulation of gamma-aminobutyric acid (GABA) in the mouse thalamus was measured after inhibition of GABA-transaminase (GABA-T) by means of systemic administration of the irreversible inhibitors gamma-acyclenic GABA (GAG, 200 mg/kg ip.) or gabaculine (150 mg/kg). After treatment with reserpine and alfa-methyltyrosine, the accumulation of GABA in both thalamus and cerebellum was decreased to 73% of controls. Likewise, when gabaculine was administered locally in the thalamus, the GABA turnover in thalamus was decreased to 75% of controls. The GABA turnover following an i.p. injection of apomorphine (0.5-1.5 mg/kg) was decreased to 82% or 67% of control, after systemic GAB or gabaculine, respectively.

Results of ongoing microdialysis studies will be reported. The data are discussed in relation to the proposed existence of positive and negative cortico-striato-thalamo-cortical feedback loops.

486.11

NEUROTENSIN AND FOS IMMUNOREACTIVITIES IDENTIFY TWO SUBPOPULATIONS OF STRIATAL NEURONS FOLLOWING ACUTE 6-HYDROXYDOPAMINE LESIONS AND REPERFUSION ADMINISTRATION. Judith S. Brog* and D. Z. Zahm, Dept. of Anat. and Neurobiol., St. Louis University School of Medicine, St. Louis, MO 63104

It was previously reported that following dopamine (DA) D2 receptor blockade or DA-depletion, neurtensin (NT) immunoreactivity (IR) is elicited in at least two distinct subpopulations of rat striatal neurons. Colocalized Fos-IR, interpreted as an indicator of enhanced neuronal activity, was found in only one of these subpopulations following D2 blockade (Neurosci. 57: 649-60, 1993). In this study, the degree of colocalization of Fos- and NT-IR in striatal neurons in response to acute midbrain 6-hydroxydopamine (6-OHDA) lesions and reperfusion administration was studied. It was observed that a subpopulation of smaller neurons exhibiting light NT-IR and frequent colocalization with Fos-IR was predominant after reperfusion administration, mainly in the dorsolateral quadrant of the striatum. Another subpopulation of larger, intensely NT-immunoreactive neurons that was rarely colocalized with Fos-IR was observed following all the drug treatments, but was present almost to the exclusion of the smaller cell type three days following 6-OHDA lesions, mainly in the dorsomedial and ventrolateral portions of the striatum. The present data are consistent with two subpopulations of striatal neurons that accumulate NT following DA depletion. The possibility is considered that one subpopulation accumulates NT in response to coordinate increases in neuronal activity and NT synthesis, and the other as a result of decreased release. Support: NIH NS-23805, T32 NS-07254, and the United Parkinson Foundation.

486.12

DIFFERENTIAL MODULATION OF DOPAMINE D1 AND D2-RECEPTOR BINDING IN RATS TREATED WITH A CLASSICAL NEUROLEPTIC, L. Liminga*, M. Curini, A.E. Johnson, L. Kilgarr, L. Hillers, A. Hjort, L.M. Gunie, and F.-W. Wiesel, Psychiatry Department, Ullrerker Hospital, Uppsala University, S-75117 Uppsala, Sweden.

Classical neuroleptics are often employed in the treatment of schizophrenia. These compounds are known to act on the dopaminergic system primarily by blocking dopamine (DA) D2-receptors. In the following experiments, we evaluated the consequences of exposure to the classical neuroleptic, fluphenazine decanoate (FLU), on DA D1- and D2-receptor binding in several regions of the basal ganglia. Adult female Sprague-Dawley rats were treated either chronically or acutely with FLU (30mg/kg) or vehicle. Animals were killed by decapitation and brain sections through the striatum (STR), entopeduncular n. (EP), subthalamic n. (STh) and substantia nigra (SN) were collected and processed for receptor autoradiography. D1-receptors were labelled with [3H]-SKF38398 in the presence or absence of unlabelled SKF38390 and D2-receptors were labelled with [3H]-NACQ298, a Eticlopride. Analysis of autoradiograms showed that in vivo exposure to FLU significantly decreased specific [3H]-NACQ298 binding up to 70% relative to controls in all brain regions measured probably by competing with [125I]-NACQ298 in vitro. With regard to D1-receptors however, FLU treatment decreased [3H]-SKF38398 binding in the dorsal STR (35%), but increased binding in EP (38%) and was without effect in the SN. In vitro competition assays performed on sections from untreated rats showed that FLU is a potent competitor of binding at both receptor subtypes with an affinity of about 10M for D1-receptors in the dorsal STR and EP and 2.5M for D2-receptors in the dorsal STR and SBs. These results suggest that although FLU is an effective competitor of both D1 and D2 compounds in vitro, it acts primarily as a D2 antagonist in vitro. These data suggest that for ligands with high affinity for both D1 and D2 receptors in vitro, these compounds may act primarily at D2 binding sites in vivo. Supported by grant #8318 from the Swedish MRC.

486.13

ELECTROPHYSIOLOGICAL EFFECTS OF A CANNABINOID ON NEURONS IN THE SUBSTANTIA NIGRA PARS RETICULATA. A.S. Miller* and J.M. Schrier, Schrier Research Laboratory, Department of Psychology, Brown University, Providence, RI 02912.

Binding studies have demonstrated that cannabinoid receptors are densely distributed in the outflow nuclei of the basal ganglia and are especially abundant in the substantia nigra pars reticulata (SNR). Cannabinoid receptors in the SNR have been localized on the striatonigral terminals. Single unit electrophysiology was used to explore the role of cannabinoid receptors by depleting the SNR. Intravenous or intraperitoneal injection of WIN 55,212-2 in anesthetized rats produced a significant but modest increase in the basal firing rate of SNR neurons. A second set of experiments was performed in order to assess whether this increase represented a disinhibition of SNR activity. Striatal stimulation produced a brief inhibition of neural activity in the SNR. WIN 55,212-2 (up to 1 mg/kg iv.) dose-dependently reversed the inhibition of cell firing in the SNR which was produced by striatal stimulation. These results suggest that cannabinoid receptors on the striatonigral terminals may regulate movement by disinhibiting the activity of SNR neurons, perhaps by inhibiting the release of GABA into the SNR.

486.14

PRE- AND POSTSYNAPTIC MEASURES OF SHORT-TERM SYNAPTIC PLASTICITY AT THE CORTICOSTRIATAL SYNAPSE OF THE AGED RAT. X. Ou* and J. P. Walsh, Andrus Gerontology Center & USC Program in Neuroscience, University of Southern California, Los Angeles, CA 90089-0191.

The corticostriatal synapse of the aged rat expresses a decrease in paired-pulse facilitation, without an associated change in short-term synaptic depression (Ou and Walsh, 1994). It is not known, however, whether age affects the kinetics of recovery from synaptic depression. Synaptic depression was induced at the corticostriatal synapse by delivering 15 stimuli (ISI of 50 ms) to the corpus callosum in an in vitro brain slice. Recovery from the depression was monitored by a test stimulus occurring 0.2 to 5 seconds after conditioning. Our data indicate that there is a significant difference between the two-age groups in the kinetics of recovery from synaptic depression with aged cells showing slower recovery from depression than young cells.

We next examined the effect of age on the contribution of NMDA receptor activation to the synaptic response. Paired synaptic responses (ISI of 60 msec) were evoked before and after addition of the NMDA receptor antagonist APV (30 μM) in Mg2+-free ACSF. Subtraction of synaptic responses evoked under these two conditions indicates that neurons from aged rats expressed a decrease in the NMDA component of the synaptic response. All slices were treated with 20 μM bicuculline to block GABA_A receptor mediated inhibition.

Research was supported by a grant from the NIA (5 P01 AG0979).
486.15 EFFECTS OF 5-HT1A and D1 RECEPTOR ANTAGONISTS ON THE INDUCTION OF STRIATAL JUN B AND FOS-LIKE IMMUNOREACTIVITY BY DEXEFENFLURAMINE. A.M. Gardner*, R. Morralia², and A.M. Graybiel².


The indirect serotonergic agonist fenfluramine (20 mg/kg, i.p.) induces Fos-like immunoreactivity (FLI) in the caudoputamen (CPu) and the caudal striatum (SMA) of the rat. In order to examine the role of different striatal subregions and other brain regions, we administered 5-HT1A and D1 receptor antagonists (Richard et al., Brain Res., 594 (1992) 131-137). However, fenfluramine is a racemate in which the d-isomer (d-fen) acts on the serotonergic system by increasing the (extracellular levels of serotonin (5-HT) as a result of vesicular storage (Saramago and Garattini, In Nutrition and The Brain, Vol.5, Raven Press, New York, 1990), whereas the l-isomer increases the turnover of dopamine (DA) in rat CPu (Invernizzi et al., Eur.J.Pharmacol., 129 (1986) 9-15). Here, the induction of rat CPu and d-fen protein was studied in rats 2 hrs after administration of d-fen (0.5, 10, and 40 mg/kg, i.p.) and induced a dose-dependent increase in both Fos-like and Jun B-like immunoreactivity (FLI, Jun B-LI) in the CPu, and in other brain regions included the central amygdaloid nucleus and layer 4 of the somatosensory cortex. The CPu induction of FLI was dose-dependently and, as caudal levels, in localized dorsal and ventral zones. The induction of F-LI and Jun B-LI by d-fen (40 mg/kg) was attenuated in CPu, but not in the cortex, by treatment with low doses (0.2 and 0.3 mg/kg, i.p.) of the D1 receptor antagonist SCH 23390. Furthermore, the induction of F-LI and Jun B-LI by d-fen (40 mg/kg) was attenuated in cortices, but not in CPu, by prior administration of a drug known as the most effective 5-HT1A receptor antagonist, methiothepin. The latter drug was administered at a dose (10 mg/kg) that has been shown to block d-fen effects on brain 5-HT metabolites (Gardner et al., Brain Res., 581(1992)67-74). Our results suggest that an indirect 5-HT agonist d-fen may stimulate FLI and Jun B-LI in the rat striatum at least in part indirectly through striatal DA release, leading to an activation of potent D1 receptor receptors.

486.16 FOS-LIKE IMMUNOREACTIVITY IN BASAL GANGLIA OUTPUTS FOLLOWING ADMINISTRATION OF DOPAMINE AGONISTS. David Witterhake* Dept. Psych., Univ. Illinois at Chicago, Chicago, IL 60609.

The pars reticulata of the substantia nigra (SNpr) and the entopeduncular nucleus (EPN) tonically inhibit cells in a number of structures including the lateral habenula, the pedunculopontine region and certain thalamic nuclei. Many workers have theorized that stimulation of dopamine receptors leads to inhibition of cells in the SNpr and the EPN which, in turn, results in a disinhibition of neurons in the systems listed above. Since many neurons respond to excitation by expressing the immediate early gene c-fos, we attempted to evaluate this model of basal ganglia organization by using immunocytochemistry to examine the distribution of Fos like immunoreactivity following systemic injections of either amphetamine (5 or 10 mg/kg of amphetamine acid (2.5 or 5 mg/kg) or apomorphine (4 mg/kg). Following all of these treatments, many labeled neurons were observed in the lateral portion of the lateral habenula, in the ventromedial thalamic nucleus and the rostral pole of the ventrolateral thalamic nucleus and in the pedunculopontine region. In subjects with unilateral 6-OHDA lesions of the ascending dopaminergic pathways, injections of amphetamine resulted in labeling of all of these structures on the intact, but not the denervated side. Simultaneous processing of tissue for NADPH-diaphorase revealed that staining in the midbrain tegmentum was concentrated in the so called "midbrain extrapyramidal area" just medial to the cholinergic cells of the pedunculopontine nucleus proper.

486.17 STIMULATION OF ADRENALINE A2 RECEPTORS BY CGS 21680 INDUCES C-FOS EXPRESSION IN THE STRIATUM OF DOPAMINE DENERVATED RATS. Morelli M.*, A. Pinna, G. Di Chiara Dept. of Toxicology Univ. of Cagliari (Italy).

The induction of the early-gene c-fos after the adenosine A2a receptor agonist CGS 21680, was studied in the striatum of normal rats or after a unilateral 6-hydroxydopamine (6-OHDA) lesion of the dopaminergic nigrostriatal neurons. CGS 21680 (5 mg/kg) induced c-fos expression in the 6-OHDA lesioned striatum while in normal rats CGS 21680 failed to induce c-fos expression even at doses of 10-20 mg/kg. The dopamine D1 agonist quinpirole reversed CGS 21680-induced c-fos expression, while blockade of muscarinic receptors by scopolamine partially prevented c-fos expression in the dorsolateral part of the striatum. The results are consistent with the coexistence of A2a and D1 receptors on striatal neurons and with their opposite influence on adenylyl cyclase. However the data also suggest that stimulation of cholinergic transmission by CGS 21680, might play a role in the induction of c-fos expression.

487.1 VESTIBULOCOCCULAR REFLEX PHYSIOLOGY


GABAergic pathways originating from the medial and descending vestibular nuclei (DVN, MVN) as well as the nucleus prepositus hypoglossi convey vestibular information to various division of the inferior olive. Previously we have demonstrated a topographic representation of both the vertical semicircular canals and utricular otoliths in one of the subdivisions of the inferior olive, the b-division. In the present experiment we have characterized the inputs, projections, as well as the encoding characteristics of neurons in another subdivision, the dorsal medial cell column (DMCC). We have recorded extracellularly from dopaminergic neurons in the substantia nigra of anesthetized rabbits placed in a three axis rate table, permitting static and sinusoidal vestibular stimulation, as well as the determination of a vestibular null point. All neurons were driven by static or sinusoidal rotations about the longitudinal axis. These neurons responded in phase with contralateral side-down head position during sinusoidal stimulation. Measured null points did not correspond to the orientation of pairs of vertical semicircular canals. These neurons appeared to be driven primarily from the utricular otoliths. These vestibularly responsive neurons were located in the dorsal half of the DMCC. An immunohistochemical stain for glutamic acid decarboxylase demonstrates that the DMCC, as well as the b-division is innervated by GABAergic fibers originating from the substantia nigra. The combination of high pass (2-4 µ, 30%) into both the nucleus-uvula and floculus demonstrates that the dorsal half of the DMCC projects to the nucleus, whereas the ventral half projects to the floculus. In sum, the DMCC accounts for some of the otolithic input to nucleus-uvula and may contribute to the topographic modulation of postural control about the longitudinal axis.

487.2 NEURAL RESPONSES TO VESTIBULAR PARADIGMS IN THE MACAQUE NODULUS. A.F. Rosenberger* and C.A. Studer, Dept. of Otolaryngology, Univ. of Pittsburgh, Pittsburgh PA 15213.

The goal of this study is to investigate the involvement of the nodulus in vestibular processing by characterizing (i) inputs and output signals, and (ii) responses to different vertical axis rotation (OVAR). Both granular layer units (GLUs) and peduncle cells (PCs) were recorded from the cerebellar cortex of an awake, trained, head-fixed monkey during passive vestibular stimulation in both horizontal and vertical planes, and during eye movements. Most neurons were sensitive to vestibular stimulation. Of these neurons that were tested in three orthogonal planes, the vast majority were sensitive to movement in a vertical plane whereas few neurons responded exclusively to yaw rotation. GLUs that responded to vertical motion were sensitive to head velocity (indicating canal or dynamic otolith input) or to static tilt (indicating otolith input). Most responsive PCs were sensitive to some combination of vertical head velocity and tilt, which in some cases, depended on the particular stimulus paradigm. Rotation of the subject resulted in convergent signals originating from multiple types of vestibular afferents. PCs were recorded during OVAR, which presumably involves velocity storage. Some PCs responded only to a modulated firing rate related to instantaneous direction of tilt, or both. The peak firing rate of units that had a modulated response component corresponded to different tilt directions. Some PCs responded to yaw rotation, with a constant rate in firing rate, a modulated firing rate related to instantaneous direction of tilt, or both. The peak firing rate of units that had a modulated response component corresponded to different tilt directions. Some PCs responded only to a modulated firing rate related to instantaneous direction of tilt, or both. The peak firing rate of units that had a modulated response component corresponded to different tilt directions. Some PCs responded only to a modulated firing rate related to instantaneous direction of tilt, or both.
VESTIBULAR: VESTIBULOOCULAR REFLEX PHYSIOLOGY

487.3

FUNCTIONAL INDEPENDENCE OF NODULAR AND UVULAR MICROVASCULAR COMPONENTS AND THEIR ROLE IN VESTIBULAR STORAGE. R. Cohen, S. Warnek, T. Raphan, H. Reisin. Mt Sinai School of Medicine and Brooklyn College of UNY.

Tilting the gravitostrial acceleration (GIA) vector during yaw-axis vestibular or optokinetic nystagmus reorients the compensatory eye velocity vector toward the spatial vertical (axis reorientation). Two processes are associated with the time constant (damping) and generation of vertical and/or torsional components in the eye velocity vector (cross-coupling). Axis reorientation was studied in rhesus monkeys following decerebration lesions that spared the superior vestibular nuclei. Tilt-induced velocity storage, reducing the horizontal time constant from 1.9±2.1s to 0.4±0.2s, without affecting the VOR gain. This abolished eye velocity reorientation, showing that the reorganization is not due to the ability to control the velocity of the rotation. Pre- and post-transected animals showed no difference in the time constant of eye velocity storage. The GIA vector was tilted in two paradigms: OKAN with static head tilt, centrifugation and dynamic head reorientation during postural changes. The superior vestibular nuclei were not affected by medial sectioning, showing that the ability to control the eye velocity vector is intact. This study is supported by grants from the Whitaker foundation and the Beckman Institute.

487.4


We investigated the effects of aminglycoseide-induced hair cell loss on the spontaneous activity and responses of anterior semicircular canal afferents in chicks. Aminoglycosides are known to selectively damage hair cells and have been proposed as a useful tool to study the role of the inner ear in the maintenance of balance. Previous studies using aminglycoseide have shown that the spontaneous activity and responses to mechanical stimulation of hair cells in the anterior semicircular canal are reduced by 60-70% and 30-40%, respectively. In this study, we investigated the effects of aminglycoseide-induced hair cell loss on the spontaneous activity and responses of anterior canal afferents in chicks. We found that the spontaneous activity and responses to mechanical stimulation of hair cells in the anterior semicircular canal are reduced by 60-70% and 30-40%, respectively. These results suggest that aminglycoseide-induced hair cell loss reduces the spontaneous activity and responses of anterior canal afferents in chicks.
VESTIBULAR: VESTIBULOCULAR REFLEX PHYSIOLOGY

487.9

SIGNAL CONVERGENCE AND DYNAMICS RECORDED FROM VESTIBULAR-OCULOMOTOR NEURONS IN THE ALERT CAT.  
S. J. Barette1 & D. E. Bialek1 1Dept. of Neurobiology, University of Chicago, IL 60611  

VESTIBULAR-oculomotor neurons contribute to the dynamics and spatial organization of compensatory eye movements produced by the vestibulo-ocular reflex (VOR). We have recorded the responses of 9 vestibular nucleus neurons in alert cats to investigate canal convergence, eye position sensitivity, response dynamics, and otohelic output. Neurons were classified according to latencies and specific responses to labyrinth electrical stimulation and antidromic responses to oculomotor nucleus stimulation.

Extensive spatial testing (14+ runs, 20 neurons), including several sinusoidal rotations about axes near near visual fixation, confirmed canal convergence on oculomotor-projecting neurons. Six of 9 neurons receiving primarily anterior canal input and 2 of 9 posterior canal neurons showed horizontal canal convergence (±20°). Maximum activation direction vectors of anterior canal neurons (AC) varied more widely than those of posterior canal neurons (PC). Vertical and horizontal eye position sensitivity of 28 vestibular neurons (16 oculomotor-projecting) varied from 0 to 31 spikes/sec/° and generally corresponded to vertical and/or horizontal vestibular sensitivity, with some striking exceptions. During sinusoidal rotations at low frequencies, subtraction of the eye position component from the total neuronal response advanced the response phase.

Responses of 26 neurons to sinusoidal rotations were nearly in phase with velocity (90° ± 18° s⁻¹ at 0.5 Hz) with advanced phases at high (129° ± 10° at 4 Hz) and low (horizontal canal, 104° ± 21°; PC, 114° ± 19°; AC, 78° ± 16° at 0.05 Hz) frequency extremes. Comparison of responses to horizontal and vertical axis pitch rotations at 0.05 Hz revealed modest phase changes for some neurons (13° to 27°) while others had large phase shifts (+91° to +98°) indicating otohelic output. Supported by EY07342, DC05599.

487.11

CONNECTIONS BETWEEN THE SACCULAR NERVE AND NECK EXTENSOR AND FLEXOR MOTONEURONS IN THE DECEMBRERATE CAT.  
H. Sato1, M. Imagawa1, M. Sasaki1, H. Begam1 and Y. Uchino1 1Dept. of Physiol., Tokyo Medical College, Tokyo 160; 2Dept. of Anat., Niigara Medical School, Tokyo 113  

The saccular (SC) receptors in the otolith organs are sensitive to vertical linear acceleration of the head. We studied the sacculo-neck connectivities by means of intracellular recording from the extensor and flexor motoneurons in C1/C2-sacral segments. Bipolar tungsten electrodes (inter-electrode distance, 0.8 mm) were fixed in place on the SC nerve; the other branches of the vestibular nerve were transected. Dorsal root (DR) and external longitudinal capitis (LC, flexor) motoneurons in C2-C3 were identified antidromically. Stimulation of the SC nerve evoked excitatory postsynaptic potentials (EPSPs) in bilateral DR motoneurons. The latencies of EPSPs ranged from 2.1 to 3.6 ms (2.5 ± 0.4 ms; mean ± SD, n=18) ipsilaterally and from 2.7 to 4.0 ms (3.1 ± 0.4 ms; mean ± SD, n=13) contralaterally. The results demonstrate the presence of reciprocal connections from SC to extensor and flexor muscles which may play an important role in the body under resting condition and during vertical linear acceleration.

487.13

LINEAR AND ANGULAR MOTION MAPPED INTO THE VESTIBULAR NUCLEUS.  
J. E. Hally1, B. Boyd and G. McCullom 1R.S. Dow Neurological Sciences Institute, Portland, OR 97209 and Oregon Health Sciences Univ., Portland, OR 97201  

Cells in the vestibular nuclei discharge in relation to motion factors including angular head velocity, linear head velocity or acceleration, the position and/or velocity of the eyes in orbit during ocular tracking and gaze hold, and events associated with eye movement. Each responding cell discharge either is in relation to a single factor or to a certain combination of two or more factors. The number of possible combinations of converging factors grows exponentially with the number of factors considered. The ensemble response across the vestibular nuclei during head and/or eye movement reflects the multitude of combinations of convergence.

We have developed a formal framework that identifies patterns of convergence within the vestibular nuclei and relates these patterns to ensemble response during movement. Each movement of the head maps formally to a certain status of the sensors, one relevant set of sensors being the hairs of the semicircular canals and otolith organs. Deflection of the semicircular hairs evokes a range of primary afferent responses, reflected in the afferent's sensitivity to velocity and/or acceleration. Convergence within the vestibular nuclei determines the ensemble response of secondary vestibular neurons. In the framework, we relate the identified patterns of convergence with the full map from head movement to discharge within the nuclei, thus identifying patterns available to the nervous system for responses such as the vestibulococular reflex, the vestibulocollidal reflex, and self-motion perception.

487.10

PVP CELLS RECORDED DURING VERTICAL VISUAL-VESTIBULAR INTERACTION, IN THE ALERT SQUIRREL MONKEY LD Dimangencio, Y Zhuang and S.M. Hightower 2Dept. of Otolaryngology and Program in Neuroscience, Washington Univ. Sch. of Med., St. Louis, MO 63110  

Extra cellular records were taken from the region of the medial (MVN) and ventral-lateral (VLVN) vestibular nuclei in the alert squirrel monkey. Responses were recorded during (i) visual following (of a full-field optokinetic drum), (ii) VOR in the dark, (iii) VOR in the light, and (iv) VOR suppression. Two types of neurons were identified, namely those that had up-eye position and down head velocity sensitivity, and those that had down eye position and up head velocity sensitivity. Both types paused for saccades and are thus classified as classic position-vestibular pause (PVP) neurons, previously reported. We conclude that some MVN & VLVN PVP cells receive input from the anterior canal (AC) and others from the posterior canal (PC). Therefore it appears that there are two components of the 3 neurons from the AC to the 3rd nucleus: 1. via the superior vestibular nucleus that conveys head and eye velocity information and a second via the MVN & VLVN conveying PVP type of information to the 3rd nucleus. The extent of flocculus control of each of these two pathways is under investigation.

487.12

VESTIBULAR INPUTS TO THE DORSAL Y GROUP NUCLEI, IN THE SQUIRREL MONKEY. P.M. Blazquez, A.M. Pertaslis and S.M. Hightower 1Dept. of Otolaryngology, Washington University, St. Louis, MO 63110  

Dorsal Y group (Y) neurons in the squirrel monkey carry visual following and vestibular signals, and the nucleus has been implicated in adaptation of the vertical VOR (Pertaslis et al 1993). Y neurons are monosynaptically inhibited by the flocculus; pharmacological inactivation of flocculus removes the visual following signal but spares a head velocity signal to Y cells. To examine the origin of this signal, we studied responses of Y cells to electrical stimulation of the labyrinths in alert squirrel monkeys.

Stimulating electrodes were implanted in the labyrinth bilaterally in two animals. Y cells were recorded extracellularly in the alert animal and identified by their characteristic response patterns during visual following and visual-vestibular interaction paradigms, as previously described (Pertaslis et al 1993). The responses of cells to electrical pulse stimulation of the labyrinths examined. Peristimulus time histograms revealed that the vast majority of Y cells were activated at diatonic latencies following stimulation of either (both) labyrinths.

Taken together with previous evidence, our results suggest that the Y group, an immediate pontomesencephalic center, is a site of interaction between vestibular signals from both labyrinths and flocculus output signals. The interneuron between the primary afferent and the Y group probably lies in the vestibular nucleus. Preliminary anatomical data indicate that the Y group receives a projection from the superior vestibular nucleus.

487.14

A COMPARISON OF THE RESPONSES OF RAT AFFERENT VESTIBULAR NEURONS AND VESTIBULAR NUCLEI NERVE CELLS EVOKED BY SINEWAVE POLARIZATION OF THE LABYRINTH. O.-J. Grüsser* and J. Kleins, Department of Physiology, Freie Universitat, 14195 Berlin, Germany  

Sine wave galvanic stimulation (0.1-100 Hz, 2-200 uA amplitude) was applied in pentobarbital-anesthetized pigmented Norwegian rats. The responses of Ggl. Scopae nerve cells were uniform and independent of their peripheral inputs (semi-circular canals, horizontal canals). Within the vestibular nuclei complex (VNC) there was differentiation: (a) Negative ipsilateral labyrinth polarization led to an activation of units driven by one or two SC inputs or by SC- and O-inputs. (b) Some VNC neurons were activated by contralateral negative labyrinth polarization. (c) Some neurons responded with a slow delayed activation to ipsilateral, some to contralateral negative polarization.

The neurons of category (a) exhibited similar response characteristics as Ggl. Scopae neurons tuned up to a frequency range between 60 and 100 Hz. Owing to the lower spontaneous discharge rates of VNC neurons, however, non-linear response components that consisted of a "silencing" during the positive phase of ipsilateral labyrinth polarization tended to emerge at lower stimulus frequencies. The lowest thresholds at 1-2 Hz galvanization did not differ significantly from those of the afferent fibers (<5 uA). The convergence of many fairly uniformly responding fibers appears to determine the response characteristics of this subgroup of VNC cells. Sine wave galvanic stimulation of the labyrinth is a powerful tool to synchronize vestibular input (Supported in part by a BMFT-grant).

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488.1 OFFSET OF HUMAN SMOOTH PURSUIT: EFFECTS OF TARGET VELOCITY, POSITION, AND PRESENCE. P. Pole and H. J. Wyatt. SUNY College of Optometry, New York, NY 10010.

We have been studying the offset response of the pursuit system as part of an effort to develop some of the system's dynamic features. Methods: Subjects observed a target moving horizontally at 15 deg/sec. When pursuit velocity became steady, the target either: (1) suddenly disappeared; (2) jumped to a different target velocity and feedback becoming 0 (target stabilized at the focus); or (3) stepped to a retinal position 3 deg ahead of the focus with target velocity of 0 and feedback of either 0 (target stabilized 3 deg from focus), -0.2, -0.4, or -1 (target fixed in space). Results: When the target disappeared, the eye decelerated rapidly (time constant T = 0.1s), but with the target stabilized at the focus, deceleration was slower (T = 0.6s). With the target stabilized 3 deg ahead of the focus, the eye continued at moderate velocity, a response to target position (since target retinal velocity was zero). As feedback increased from 0 to -1.0, eye deceleration systematically increased. Modelling and Conclusions: All of the experimental results can be simulated by a model with target velocity and position inputs, and an internal positive feedback loop. A key feature of the model is that the positive feedback loop is enabled by the presence of a visual target. Thus, long-time-constant offset reflects the action of the positive feedback loop, whereas short-time-constant offset is a result of a loss of positive feedback following target disappearance. The model also suggests that the systematic change in deceleration with increasing feedback depends on the effects of a pursuit position mechanism — the same mechanism that drives the eye when the target is stabilized 3 deg ahead of the focus.

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488.3 CRANIOPTIC SMOOTH PURSUIT DEFICITS IN HUMANS. M. J. Morrow. Olive View U.C., Department of Neurology, Los Angeles, CA 91342.

Nuclear circuits arranged in a head-centered (cranioptic) framework influence smooth pursuit eye movements. Cranioptic nuclear motor organization is demonstrated in patients with acute cerebral hemispheric damage causing ipsilateral gaze deviation. These patients typically cannot generate pursuit movements across the orbital midline, away from the side of the lesion. Smooth pursuit was recorded from the frontal and central retinal lobe infarction. By comparing responses to target motion within the right and left gaze fields, cranioptic pursuit deficits were quantified. Responses were compared in rightward and leftward directions to identify directional pursuit asymmetry.

One patient had left hemispheric infarction with right spatial neglect and left gaze deviation. He could not make any eye movements across the orbital midline to the right, but smooth pursuit had lower velocities to the left within the left gaze field. Three patients with similar gaze deviation or nuclear motor region generated worse smooth pursuit in the gaze field contralateral to cerebral damage; each of these patients had lower velocities of pursuit for target moving in the ipsilateral direction. Cranioptic pursuit deficits are distinct from pursuit impairment based on the direction or retinal locus of motion. These deficits comprise a spectrum in which the most severe examples are associated with complete inability to generate smooth pursuit in the contralateral field of gaze. The cranioptic pattern of impairment implies that orbital positions are taken into account by cerebral mechanisms that govern smooth pursuit in humans.

488.4 ADAPTATION OF OPEN-LOOP SMOOTH PURSUIT RESPONSES IN THE RHESUS MONKEY. MEIERDINK, KALB, AND STEPHEN Q. LISBERGER. Dept. of Physiology and Keck Center for Integrative Neuroscience, UCSF, San Francisco, CA 94143.

During the initiation of smooth pursuit eye movements, there is a clear relationship between eye acceleration in the first 100 ms (the open-loop period) and target velocity. We have developed an adaptation paradigm for changing the eye acceleration response to a particular velocity. Adapting responses to track a target to a velocity that began to move at an adapting velocity, but accelerated or decelerated, after 100 ms, to a higher or lower velocity. Thirty minutes of adaptation caused a change in the acceleration of the target that began to move at an adapting velocity, but accelerated or decelerated, after 100 ms, to a higher or lower velocity. Thirty minutes of adaptation caused a change in the acceleration of the target.

The largest change in eye acceleration occurred in the interval 50-60 ms after the onset of pursuit, with less change occurring in the initial 0-30 ms interval. The adaptation decreased the eye acceleration along the horizontal direction, but did transfer in an unexpected way to other target velocities. With a stimulus that started at 10 deg/sec and accelerated to 30 deg/sec, the greatest changes occurred at test velocities lower than the adapting velocity. This paradigm will allow us to study neuronal changes that underlie adaptation of a relatively well-defined voluntary behavior that requires the cerebral cortex. Supported by NIH grant EY02878.

488.5 TWO DIMENSIONAL SMOOTH PURSUIT EYE MOVEMENTS DURING TRACKING OF DOUBLE SINE WAVES IN MONKEY. RE Kuffler, H.C. Lacey. ME Gerard & SJ Takikawa. Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

We examined smooth pursuit in the rhesus monkey along simple 2D trajectories by moving a back-projected laser spot successively along horizontal and vertical axes. Component waves had a maximum ratio of 2:3 and were equated for velocity. Stimulus and sum-of-sine tracking along the single axes were studied at the same frequency.

A computer generated contant eye position via a scalar search. Gain and phase were computed by correlating a reference to the stimulus.

Above about 0.6 Hz, the gain of all components decreased with increasing frequency. Phase differences were always less than 60° indicating good predictive pursuit. There were several components initially shown in the interactions between components on both the same axis and on opposite axes. (1) The addition of a higher frequency component reduced the gain of a component presented alone. (2) Reductions were greater for components added to the same as well as the target frequency. We found that high frequency components exceeded the target and low frequency components. (3) Horizontal and vertical waves were larger than the target velocity for component, as well as the target and low frequency components.

The study showed significant effects of components presented within 15 and same frequency. These effects indicate nonlinear interactions between components both within and across axes, and suggest distinct roles for high and low frequency components of the eye movement system. Other experiments reported similar data for 10 pursuit in humans. We also studied combinations between kinematic variables and tracking performance. Corrective saccades were mostly clustered around points of peak velocity. Pursuit gain was correlated with measures of peak velocity, acceleration and jerk. This explains why pursuit was better for cross-axis combinations that appeared subjectively smoother than the same components acting along the same axis. (Supported by grant MH 48188)

488.6 TWO DIMENSIONAL SMOOTH PURSUIT EYE MOVEMENTS DURING CIRCULAR TRACKING AND PERTURBATIONS IN MONKEY. H.C. Lacey, RE Kuffler, ME Gerard, SJ Takikawa. Dept. Physiology, Northwestern University Medical School, Chicago, IL 60611.

We studied smooth pursuit along three trajectories: circles, circles with occasional perturbations along horizontal or vertical midlines, and horizontal and vertical sinusoids. Reliable behavior developed after repeated sessions with reward delivered contingent upon correct fixation. Horizontal and vertical eye and target velocity curves were computed using a least squares error fit after removing all the saccades. Eye velocity gain was defined as eye velocity divided by target velocity. Eye velocity gain and phase response to a 5° circular trajectory with a frequency range of 0.2 to 1.8 Hz was examined. Gain and phase curves of horizontal and vertical components initially showed in the experiments were less than 60°, measured by decreasing gain and increasing phase lag at higher frequencies. Comparison analyses of horizontal and vertical sinusoidal pursuit showed similar trends. In both cases, corrective saccades were preferentially observed in a particular direction of horizontal and vertical velocity points, especially for the sinusoids. Because a number of kinematic variables including peak velocity and acceleration vary systematically during these tracking behaviors, this result was restricted to particular sinusoidal frequencies. Geckell and Tamminger (1984) have obtained similar gain and phase results in humans. Horizontal and vertical perturbations produced consistent corrective saccades after a delay of 130-180 ms. After perturbations and before corrective saccades, pursuit followed the curved trajectory of the non-perturbed waveform. This continuation of pursuit is caused by the prediction of the circular waveform instead of the maintenance of velocity in a single direction. Tracking along the perturbed axes showed attenuation in amplitude at all frequencies. However, pursuit along the non-perturbed axes only showed attenuation at higher frequencies. This indicates cross-axis interactions under these conditions. (Supported by grant MH 64185)

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488.7

VISUAL TRACKING OF ILLUSORY MOTION IS AFFECTED BY TARGET WAVEFORM. A. Z. Zivotovska, I. Avberšek-Heller, C. W. Thomas, V. E. Das, A. O. DiLuzio, and T. Lei. VA Medical Center and Case Western Reserve University, Cleveland OH 44106

We studied ocular motor responses to a variant of the Duncan illusion, in which the perception of motion of an object is induced by moving its frame of reference.

Using the magnetic search coil technique, we measured head-fixed and head-free tracking responses of 10 normal subjects to vertical movement of a target (laser spot). Movement of the target was synchronized to horizontal movement of a sinusoidal grid with a period of 20 X 20 deg, producing a strong illusion of diagonal motion, the horizontal component being opposite to the direction of the grid. Both visual stimuli moved ± 9.2 deg at 0.35 Hz, either sinusoidally or in square waves. In response to sinusoidal target motion, gaze always followed the target vertically, with a small horizontal component (mean gain 0.05) that was phase lagged compared with grid movement by a mean of 37 deg. When the head was free to move, it usually (85%) showed a diagonal trajectory contrary to the gridded motion (mean gain 0.32) that was phase lagged compared with grid movement by a mean of 156 deg. In response to stepping target movement, inappropriate horizontal saccades were made for 71% of steps. When these saccades were anticipatory (38%), they were always (100%) in the direction of the illusion; when not (latency >90 msec), 49% of saccades were still in the direction of the illusion. The head tracking movements in response to stepping targets were also (100%) in the direction of the illusion. Thus, ocular tracking for sinusoidal and stepping target waveforms, the former being response to actual target motion, the latter to illusionary target motion. Head tracking, however, was always an illusionary tracking of the target, irrespective of its waveform.

488.9

OTOLITH DETECTION OF ANGULAR VELOCITY IN THE VOR. B. J. M. Hess*, D. E. Aneleaki, and J. J. Sasaki. Dept. of Neurology, Univ. of Zurich, Switzerland, Dept. of Surgery (Otolaryngology), Univ. of Mississippi, Jackson MS, Dept. of Otolaryngology, Tokyo Univ, Tokyo Japan

It has been shown previously that in the presence of simultaneous dynamic otolith input the vestibulo-ocular reflex (VOR) represents more faithfully head angular velocity in the low frequency range (Rade and Baker 1988; Tomko et al. 1998). These observations have raised two yet unresolved questions. First, do these characteristics of the otolith system represent a fixed or a learning property? Second, what is the functional significance of such a property? We have addressed these questions by examining the otolith contribution to the VOR in the absence of sinusoidal low frequency oscillations of the phase plane, using sinusoidal low frequency oscillations (0.1-0.01 Hz, ± 50°) and constant velocity-off-vertical axis rotations (0.5-5°/s).

Our results in the intact animals indicate that the low frequency VOR enhancement during simultaneous dynamic otolith stimulation reflects the sensitivity of the otolith system to head angular velocity. A masking observation was made in canal plugged animals: Acutely after canal plugging, the otolith contribution to the VOR was unchanged, however, it progressively decreased thereafter. Two months after the canal plugging, the low frequency and steady-state responses to sinusoidal and constant velocity off-vertical axis rotations were completely abolished in the plane of the plugged canals. Our findings in canal plugged animals do not support the current belief that the main function of the canal/otolith interactions through the velocity storage mechanism is to improve the low frequency VOR dynamics. Rather the low frequency dynamic otolith contribution to the VOR could reflect the inertial representation of vestibular angular motion signals in the velocity storage network (Aneleaki and Hess 1994).

488.11

TEMPORAL CONSTRAINTS ON THE MECHANISM OF LEARNING IN THE VOR. Jennifer L. Raymond* and Stephen G. Lisberger, UCSF Dept. of Physiology and Kirk Cherry, UC San Diego School of Medicine, San Francisco, CA 94143

Changes in the amplitude and dynamics of the vestibulo-ocular reflex (VOR) can be induced by the association of vestibular and visual (image motion) stimuli. We examined the dependence of these learned changes in the VOR on the temporal properties of the sensory signals used to induce learning. First, we studied for 3 hours with sinusoidal vestibular and visual stimuli at a single frequency from 0.5 to 10 Hz (±10°/s). The stimuli for adaptation were provided by passive head turns either with or without sinusoidal visual stimulation that mimicked the effects of magnifying or minimizing spectra. Adaptation with 0.5 to 5 Hz for 3 hours produced changes in the VOR, while maintaining the gain and phase of the VOR at the adaptation frequency, to a similar degree as with a step change in the VOR. Changes in the gain and phase of the VOR due to adaptation with 0.5 to 5 Hz for 3 hours were evident 1-2 days after adaptation. The changes in the VOR were small relative to the change in the phase of the VOR, while compared to the simultaneous onset or delayed visual stimulus onset. These temporal constraints on the stimulus that maximized these learning effects are likely to identify the neural mechanism(s) of plasticity in VOR pathways. Supported by the NASA Space Biology Research Associate Program and NIH grant EY01198.

488.8

SPATIOTEMPORAL VOR ADAPTATION AFTER SEMICANALICULAR INACTIVATION. D. E. Aneleaki*, B. J. M. Hess and J. J. Sasaki. Dept. of Surgery (Otolaryngology), Univ. of Mississippi, Jackson MS, Dept. of Otolaryngology, Univ. of Zurich, Switzerland; Dept. of Otolaryngology, Tokyo Univ, Tokyo Japan

Selective semicircular canal inactivation by plugging and three-dimensional eye movement recordings were used to address the following questions: 1) Is the 3D structure of the VOR matrix which relates semicircular canal inputs to oculemotor output? 2) How is the VOR matrix modified during recovery from inactivation of either of the vertical canals? To address these questions, horizontal, vertical and torsional eye movements were recorded during sinusoidal oscillations at different frequencies (0.01-1 Hz) and head orientations. The VOR matrix was computed before and after acute and acutely after canal plugging. Similarly, the evolution of the VOR matrix elements during recovery was computed from data acquired two weeks, as well as one, three and eight months after canal plugging.

The VOR matrix elements were frequency dependent. At low and high stimulus frequencies, inputs from the lateral and vertical canals contributed to torsional and horizontal eye movements. At low frequencies, however, the VOR matrix was diagonal. After semicircular canal inactivation, the VOR matrix in the mid and high frequency range changed continuously while gain and phase in the plugged canal plane progressively recovered. No recovery was ever observed at frequencies below approximately 0.05-0.1 Hz. These results suggest a frequency-selective central reorganization of vestibulo-ocular connectivity after selective semicircular canal inactivation. In addition, the limited range of the organization of the VOR in the mid and high frequency range differs distinctly from that in the low frequency range. This observation raises the possibility that the "velocity storage" was enhanced in the oculemotor system but that it might represent a distinct entity with different organization and function.

488.10

DIFFERENTIAL EFFECTS ON COMPENSATORY GAZE OF INTRAURAL (IA) LINEAR ACCELERATION IN MAN AND MACAQUE MONKEYS. S. Warren, T. Raphan, B. Cohen. Brookline, and T. J. Schabalk and C. Thyagarajan, Brookline, MA, Tufts University School of Medicine

Contributions of linear and angular vestibulo-ocular reflexes (LVOR & AVOR) to gaze stabilization were determined in macaque monkeys during centrifugation, off-vertical axis rotation (OVAR) and optokinetic after-nystagmus (OKAN). Animals were centrifuged either facing or back to motion with velocity trapezoids (40°/s² to 400°/s²) producing peak IA linear accelerations of 1.24g. Peak horizontal eye velocity (VEH) was invariant for the two orientations and beating fields remained close to the midline. Horizontal time constants (Tc) fell faster with GIA tilt (when facing than back to motion (.38s vs .69s)). This corresponded to UC asymmetries during yaw axis OKAN for low velocity stimuli with speeds decreasing before and after down and forward coupling. Dynamic parameter modification as a function of tilt was incorporated into our velocity storage model, using the OKAN Tc's from each monkey. These simulations accounted for all responses to VEH during centrifugation. Despite this, these monkeys had a direct horizontal translational LVOR during OVAR at velocities of 5°/s to 360°/s (~0.01-1Hz). Plugging the semicircular canals made animals less sensitive to yaw axis OKAN during centrifugation with similar GIA magnitudes (Warren, 1993). A direct consequence from the translational LVOR and consequent shift in the beating field was necessary to model the linear responses. The LVORS both modify the Tc's of velocity storage, reorienting AVOR coordinates, and generate direct compensatory responses to translation. Differentially weighting these two mechanisms accounts for different effects of linear acceleration on compensatory gaze in man and monkey during centrifugation. SUPPORT: EY00414, EY00867, N01RR20, P60-CN2 P60-CN240, P60-CN2340.

We used electromagnetic search coil techniques in darkness to assess at equilibrium the sinusoidally varying eye velocity (modulation) and average eye velocity (bias) of the VOR in cats during 5-32 Hz constant velocity horizontal axis pitch rotations. Modulation of vertical VOR eye velocity increased with head velocity, and was greater at all velocities for backward (nose up) than forward (nose down) rotations. Minimum modulation responses shifted toward forward rotations, occurring at +45°/s forward pitch. Slow phase eye velocity bias increased nonlinearly with head velocity and was greater for forward than backward rotations at all velocities. Minimum average eye velocity shifted toward backward rotations, occurring at -27°/s (backward).

We propose that these mechanisms are responsible for this behavior. (1) A central velocity estimate, generated from otolithic signals as proposed by others, which produces an eye velocity bias that increases with head velocity in the range tested. (2) As afferent or central "pitch" signal, generated from the rate of change of an anterior-posterior otolithic stimulus, which produces modulation of eye velocity increasing with head velocity. (3) A gravity-dependent dependent nystagmus (GDDN), as we reported in cats (NS 91), generated by a saccadic response to tilt, summing with the central velocity estimate to affect bias while also summing with the jerk signal to affect modulation of vertical eye velocity. The static position GDDN is near zero in the upright position and peaks with an upward slow phase of 180°/s tilt. During rotation the upward slow phase velocity of GDDN adds to the central velocity estimate, subtracting during backward rotation when the velocity bias is downward. Analogously the peak upward slow phase velocity of the GDDN signal at 180°/s tilt attracts from the peak modulation signal only during forward rotations when, at 180°/s, the eye velocity is downward. Thus the GDDN signal is of the correct direction and magnitude to account for the observed shifts in minimum slow phase eye velocity modulation and bias at low rotation velocities.

Supported by EY07342.

488.15 STATIC AND DYNAMIC EFFECTS OF GRAVITO-INERTIAL ACCELERATION (GIA) ON SPATIAL ORIENTATION OF VELOCITY STORAGE T. Rebber*, S. Yeung, B. Cohen. Brooklyn College of CUNY, Mt. Sinai School of Medicine.

We compared axis orientation during off-axis rotation and dynamic head tilt during post-rotatory nystagmus (PRN), i.e., tilt damping, to that during OKAN. We tested rotation from pitch to roll/yaw and roll to pitch/yaw. Absence of the latter was a key test in model-based studies of OKAN axis reorientation (Raphan & Stern, 1991). Monkeys were rotated off-axis at 10°/s & 40°/s to velocities of 200-400°/s, tilting the GIA vector from 17°-72°. Orientation vectors were computed using a model-based approach. During acceleration, the GIA vector had pitch and roll components and both pitch and roll cross-coupled eye velocities were generated. At constant velocity, the GIA pitch component remained, inducing continued pitch eye velocity that decayed with a 5-6s Tc. This was close to the yaw Tc. The roll component of the GIA dropped rapidly and roll velocity decayed with a short time constant (Tc = 1 s). Off-axis rotations of monkeys such that the GIA vector was solely in the yaw plane elicited no pitch-roll or pitch-roll axis reversing nystagmus. Yaw tilt damping during post-rotatory pitch rotation (PRN) with peak tilt velocities < 60°/s elicited no significant horizontal velocity and no pitch-roll or roll-pitch cross-coupling. Thus, the reported 'pitch-yaw' and 'pitch-roll' cross-coupling (Angelaki & Hess, 2004) is likely due to high velocity pulses along the yaw axis that charged the roll state with a small time constant which then discharged with a longer time constant when the animal was supine/prone. It is not due to additional off-diagonal terms in the system matrix. The recognition of eye velocity trajectories was the same during dynamic head movements and centrifugation as during OKAN (> 10°/s from the spatial vertical), and was predicted by dynamic parameter changes in the system matrix of velocity storage. We conclude that the yaw axis vector codes the spatial orientation of velocity storage during vestibular activation as during OKAN. Only the yaw axis vector is affected toward the spatial vertical when the GIA vector is shifted; the pitch and roll axes move with the head.

488.16 REDUCTION OF VOR TIME CONSTANT WITH MAINTAINED VOR IN A GROUP OF ELDERLY SUBJECTS. M. Dai*, B. Cohen, T. Raphan. Mount Sinai School of Medicine, Brooklyn College of CUNY.

Some elderly patients have short bilateral vestibulo-oculare reflex (VOR) time constants (Tc) but normal or high VOR gains. They generally complain of imbalance rather than vertigo. The anatomic basis for these findings is unknown. We studied 14 subjects, aged 75-93, with short VOR Tc and with high VOR gain, determined from the initial jump of slow phase velocity of velocity steps, ranged from 0.5-1.0 (mean 0.74±0.15). VOR Tc ranged from 3.5-5.0 (mean 4.2±0.6 s), compared to Tc = 15-25 s in normals. The gains of optokinetic nystagmus (OKN) induced by high field motion were normal (gain = 0.9-2.0 for 20 & 40 cycles/s), and there was no optokinetic after-nystagmus (OKAN). Subjects were rotated in a lighted earth-fixed surround for 15 sec and stopped in darkness. The post-rotatory VOR gain was the same as after they had been rotated in darkness. The post-rotatory eye velocity storage was not activated during OKN. This is in contrast to the normal reduction of post-rotatory eye velocity by 40-100% after rotation in light. The short VOR Tc and the absence of OKAN as well as the inability to reduce post-rotatory eye velocity could be modelled by a reduced torsional Tc and a deactivated velocity storage integrator. Aging is associated with degeneration of hair cells and a loss of fibers in the vestibular nerve. The selective impairment of the VOR Tc with preservation of gain may be secondary to selective hair cell or nerve fiber loss. The imbalance noted by these elderly people may be related to inactivation of velocity storage, which is important for coding spatial orientation and stabilizing gaze and posture during movement.

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489.3
HIGH FREQUENCY, PITCH AND YAW VESTIBULO-OCULAR REFLEX (VOR) IN VERY ELDERLY PEOPLE. J. L. *Petter* and Robert W. *Halmagui*. Neurology & Department of Neurology, University of California, Los Angeles, 90024.

Advanced age is known to result in a shortening of the time constant of the yaw VOR at high frequencies, resulting in reduced low frequency gain and increased phase lead. Since these conditions are not representative of physiologic head movements during everyday life, we investigated the VOR of very elderly subjects during high frequencies in pitch and yaw.

Eye movements were recorded using magnetic search coils for pitch and electro-oculography for yaw in groups (N = 9) of young subjects (mean age 30 yrs) and very elderly subjects (mean age 80 yrs). Sinusoidal whole-body rotations (peak velocity 20-30°/sec) were presented. Gain and phase of the VOR was measured as a function of frequency. The VOR was obtained from digitally sampled signals following saccadic removal. It is greater for pitch than for yaw, and gain increased with increasing frequency. Gain in the very elderly was never significantly less than that in the young. The pitch VOR gain of the elderly was actually significantly greater than that of the young at 0.8 Hz (0.46±0.04 vs. 0.36±0.11, mean ± standard deviation, P < 0.05).

Very elderly people have no functionally significant determination of pitch and roll VOR at velocities ≥30°/sec. Possible velocity saturation in the high frequency VOR of the elderly, such as occurs at low frequencies, could be yet to be elucidated and is being studied in our ongoing experiments. However, these data suggest that the rotational VOR of elderly people is normal in the important function range of frequencies and velocities.

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489.5

Following unilateral vestibular loss, high speed rotation towards the intact ear resulted in a higher gain than rotation towards the defective ear, resulting in a direction-dependent pendular response (DP) ([Gain=Int-Gain=Def]) (Gain=Gain Int)*100). This occurs because of the inability of inhibitory stimuli to decrease vestibular nerve firing rates to < 0. We examined the DP in 4 patients following unilateral vestibular nerve section, and in 2 patients with partial labyrinthectomy with absent caloric but spared vertical SCC on CT scan and compared it to 6 normal subjects. The horizontal VOR was measured by EOG during constant-velocity step rotations (20°/sec) while the head was pitched 30° up, kept level or pitched 30° down.

The DP in the control was less than 5% regardless of head pitch. The DP in patients with unilateral vestibular loss increased with increasing pitch in a manner less than expected by the degree in which the H and V SCC sense head acceleration. When the head is pitched down, head acceleration is maximally sensed by the H SCC and DP is maximum (23%). When the head is pitched 30° up 61% of the head acceleration is sensed by the H SCC. 25% is sensed by the V SCC, and there is little DP (4.2%). In the patients with spared V SCC, there is little to no DP when the head is level or pitched up.

These results suggest that rotatory cliché of head pitched up and down may be a useful technique for assessing V SCC function.

489.6

Using dual search coils we have recorded 3-dimensional eye position in normal subjects and in patients after unilateral vestibular deafferentation on the right (RUVD) or left (LUVD) side. We used two different paradigms. In a first set of experiments, subjects were exactly aligned with the axis of rotation, and then rotated at 20°/sec about an earth vertical axis to a steady state velocity of 250°/sec. These experiments produced two interesting results: first, even in normals, rotation about an earth vertical axis elicits torsional eye movements; and second, the torsional eye velocity components in LUVDs, normal subjects, and ENU mice, are different. In a second set of experiments, subjects were placed 1m out on the arm of the centrifuge, facing either toward the center of the rotation or away from it. Then they were accelerated at 10°/sec to 200°/sec. The results from eccentric rotation show the relative contributions of the saccadic canals and the otoliths, and an analysis of these experiments is presented.

489.7
THE EFFECT OF HEAD PITCH ON HORIZONTAL VOR GAIN DURING ROTATION AT HIGH SPEEDS. M.P. *Grant*, D.Y. *Toppi*, and D.S. *Zee*. Johns Hopkins University School of Medicine, Baltimore, MD 21207.

The VOR serves to maintain a stable retinal image during rapid changes in head position by producing slow-phase eye movements to offset the change in head position. Previous studies suggest that at low velocities (< 100°/sec) head pitch does not influence the gain of the horizontal VOR. We recorded horizontal VOR gain using EOG in 7 normal subjects during constant velocity chair rotations (60-300°/sec at 60°/sec) in yaw with the head pitched up or down 30°. The mean VOR gain with the head pitched down was 0.56, independent of speed. With the head pitched up, the gain decreased from 0.58 at 60°/sec to 0.40 at 300°/sec.

During the normal VOR at high velocities, central mechanisms must compensate for the nonlinear behavior of the primary vestibular afferents since the vestibular afferents are driven into inhibitory cutoff. This study demonstrates an unexplained decrease in the gain of the horizontal VOR in the head up position, suggesting the central mechanisms are not completely compensating for the nonlinear behavior. Two hypotheses which could explain our observations are: 1) Otolithic afferents may modulate the "antinonlinearity" mechanism. 2) The inputs from the horizontal and vertical semicircular canals may affect the "antinonlinearity" mechanism in different ways.

489.8

We recorded eye position during free text reading in a 48 year old man with clinically undiagnosed reading discomfor. Position traces were digitized at 200 Hz with a resolution of 1.52 arc min. Nonlinear dynamical systems analysis by the Grassberger-Procaccia algorithm demonstrated a correlation dimension (Dc) less than 3.0. In this patient, gross inspection of position and delay map traces during sinusoidal pursuit testing showed satisfactory pursuit right of the midline and saccadic pursuit left of it. This asymmetry persisted at all velocities tested.

Previous analysis of reading eye movements showed a Dc near 3.3 for 2 normal subjects, near 2.1 for a slow reader and a dyslexic, and indeterminate for a patient with acquired dystagmus. All non-dyslexic subject data showed evidence of multifractal behavior. These findings suggest that fractal analysis of unconstrained eye movements alone cannot explain the differences between normal and abnormal reading eye movements. Experiments using a forcing function and more sophisticated multifractal analysis may be more sensitive.
FAST SACCADE RESPONSES IN PARKINSON’S DISEASE

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Saccadic reaction times of 14 patients with mild to moderate Parkinson’s Disease (PD) were compared to those of 9 elderly control subjects (CS). The target appeared 8° left or right from the central fixation point at the time when the fixation point was switched off (gap 0) or 200 ms later (gap 200). An experimental session consisted of 200 trials in which directions and gap intervals were randomized. Only primary saccades were considered.

We found no significant differences in the mean saccadic reaction time between the PD and control group in either gap condition. The mean saccadic reaction time was shorter for gap 200 (PD 166 ms; CS 179 ms) than for gap 0 (PD 221 ms; CS 246 ms); this difference (gap effect) was not statistically different in between groups. There was a tendency toward shorter reaction times and a higher proportion of anticipatory saccades in the PD group. Express saccades were present in some gap 200 trials of several subjects in both groups. Therefore, we conclude that express saccades may be mediated by pathways which do not involve the basal ganglia.

In some subjects, anticipatory responses, identified by directional error, extended to longer reaction times and the entire saccadic reaction time distribution was shifted along the time axis. To allow for these effects, we proposed 1) correction of the bin value in the saccadic reaction time distribution based on the statistical estimation of the proportion of anticipatory saccades; 2) flexible boundaries for visually guided saccade types (express, fast regular, slow regular) by introducing an adaptive classification schema.

CONTROL OF POSTURE AND MOVEMENT VII

THE INFLUENCE OF TARGET MOVEMENT ON REACHING AND GRASPING IN LEUKOTOMIZED AND UNLEUKOTOMIZED ADULTS WITH SCHIZOPHRENIA

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Seven leukotomized adults with schizophrenia (LS), eight unleukotomized adults with schizophrenia (ULS), and eight healthy control (C) individuals were required to reach toward and grasp a small object that was either stationary or moving. Reflective markers were placed on the subject’s index finger, thumb, and wrist, and movements were videotaped. As expected the LS and ULS groups moved more slowly than the C group when the target was stationary. However, when the target was moving, all three groups moved faster, with the LS and C groups having the same movement times, and the ULS group having the fastest movement time. When the timing of the reaching trajectory was assessed, the LS group spent less time decelerating toward the object, indicating their movements were controlled with less precision. When grasp formation was analyzed, for the stationary condition, the maximum amplitudes of the LS and ULS groups were not different, and both were larger than those of the C group. For the moving target condition, aperture increased for all groups but was smallest for the C group, intermediate for the LS group and largest for the S group. There was actually no within subject variability in peak aperture for the LS and ULS groups in comparison to the C group, perhaps indicating a limited repertoire of motor response. These results are discussed in terms of the role of the frontal lobes in reaching and grasping, and the ability of schizophrenic individuals to use redundant information. (Supported by NSERC).

UPPER EXTREMITY RHYTHMIC MOVEMENTS: EFFECTS OF ARM POSITION AND FATIGUE ON DYNAMIC PATTERN SWITCHING AND MUSCLE COACTIVATION

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Dynamical motor patterns of the fingers have shown evidence of multistability and nonlinearity. As rate of cycling increases, loss of stability and pattern switching occur. We examined how these effects were different in whole arm movements in different forearm positions, pronated and supinated, and in the rested and fatigued states.

Subjects were asked to trace a figure-8 on a digitizing tablet using a writing cuff to stabilize pen position. Surface EMG was obtained from the pectoralis, infraspinatus, biceps, triceps, wrist flexors and extensors. Subjects cyclically increased the rate of tracing until pattern switching occurred.

The critical frequencies for pattern switching depended on the position of the forearm, the orientation of the figure-8, and the direction of movement. EMG analysis indicated that the majority of control occurred in the proximal muscles at slow speeds, but distal activity increased with speed. The analysis of the pattern switch, followed by a change of EMG activity from tonic to more phasic, fatigue decreased the critical frequencies and shortened the latency of coactivation, but does not appear to alter the relationship between those characteristics.

IMAGE PROCESSING MEASUREMENT OF EYE MOVEMENTS IN HUMANS AS A Viable ALTERNATIVE TO SCLERAL SEARCH COILS

S.T. Moore, T. Haslwanter, I.S. Curthoys, G.M. Halmagyi
Dept. of Psychology, University of Sydney, NSW, 2006.

Up to now, scleral search coils have been the only way to measure 3-dimensional eye position in human subjects accurately. The main disadvantages of search coils are the need to wear a contact lens, and the cost of the coils. To overcome these disadvantages, our group has developed an image processing technique for measuring 3-dimensional eye position “VMT”, which is based on commercially available video cameras and personal computers. Horizontal and vertical eye position are determined by tracking the center of the pupil; torsion is obtained by measuring the amount of rotation of a segment of the iris about the pupil center. We found that it is possible to express eye position accurately in a manner analogous to coils, i.e. in rotation vectors or Euler angles, if we take into account a geometrical distortion of the video image of the eye when calculating eye position. Neglecting this image distortion leads to large errors in measured ocular torsion. We present a summary of the techniques used, the effects of the correction algorithms, and show experimental data, for example Listing’s Plane, obtained with this video system.

A METHOD OF ANALYSIS TO DETECT AND CHARACTERIZE UPPER LIMB CONTROL DEFICITS IN SUBJECTS WITH STROKE

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KE Light Motion Analysis Laboratory, UNC CH, Chapel Hill, NC 27599-7153.

The purpose of this study was to develop measurements based upon dynamic systems analysis and nonlinear dynamic for the comparison of the upper limb trajectories and reciprocal limb movements and to characterize limb control deficits in subjects with stroke. Twenty five subjects with CVA (12 left, 13 right) and 25 age and gender matched non-disabled (ND) subjects were tested vertical limb movements. All subjects were videotaped for 2-D analyzing 15 times fast as possible with a stylus on a 3 inch target for 10 seconds. The trajectory of the stylus was digitized at 60 Hz and the data were 4 times upsampled through Fourier interpolation. Variables included: cycle period, peak vertical position, up and down velocities, the ratio of amplitude/velocity slopes, the ratio of acceleration to deceleration duration for upstroke and downstroke, the ratio of maximum to average velocity for upstroke and downstroke, Pearson’s r coefficient for return maps of cycle period and peak amplitude, and harmonic magnitude ratio (HMR). Subjects with CVA had lower tapping frequencies (< 0.0001) than ND subjects and exhibited spatial and temporal asymmetries between upstroke and downstroke that were markedly different than those of the ND subjects. Return maps of peak position showed significant (< 0.0001) differences in the control of successful peak vertical positions due to dominance, and to the presence of right and left brain lesions. Significantly higher values for HMR were seen for dominant limbs of ND subjects, and for ND subjects compared to subjects with CVA. High values for HMR may represent minimum jerk movements. The results of this study suggest that these analyses captured the movement dynamics of the stylus trajectory and are sensitive to subtle differences in upper limb control between dominant and non-dominant limbs in ND subjects and between involved and uninvolved limbs of subjects with CVA. Supported in part by a grant from the Foundation for Physical Therapy.
490.5
FRICCTION, NOT TEXTURE, DICTATES GRASP FORCE USED IN OBJECT MANIPULATION. Genevieve Cadoret* and Allan M. Smith, Centre de Recherche en Science Neurologique, Universite de Montreal, Quebec, Canada, H3C 3J7
A study of grasping and lifting in 10 subjects indicated a capacity to detect the friction of surfaces of a grasped object against the skin and adjust dynamic and static grip forces accordingly. Ten sandpaper surfaces, were composed of smooth or 1.0 mm raised beads spaced at 2.0 or 3.0 mm intervals etched in polyamide plastic. In addition, the surfaces were treated with lubricant (e.g. talc), or adhesive (e.g. water or sucrose) coatings which either increased or decreased the coefficient of friction. Changes in friction were associated with appropriate and inversely scaled changes in grip force. Both the dynamic grip force used to lift an object, as well as the static force used to hold it against gravity were negatively correlated (r = -0.70) with the coefficient of friction. Surface texture was only effective in changing grip force insofar as it altered the surface friction. Lubricants had less effect on the coefficient of friction of textured surfaces because of the smaller area in contact with the skin. Conversely, adhesives increased the friction of the smooth surface more than the beaded surfaces. The grip forces for all subjects reflected changes in the coefficients of friction and ignored the changes in surface texture. When coatings were used to equate different textures to the same coefficient of friction, the grip force profiles were nearly identical. This research was supported by the Medical Research Council of Canada and a fellowship from FCAR groupe de recherche sur le systems nerveux central.

490.7
DISCRETE POINTING MOVEMENTS IN ALZHEIMER’S DISEASE: EVIDENCE OF PRESERVED AND IMPAIRED MOTOR CONTROL. J.D. Faul, K. St. Leger, L. de Chazal, P. McGeer, L. McGeer, Dalhousie University and Camp Hill Medical Centre, Halifax, N.S., Canada
This study employed a kinematic analysis of visually directed upper limb movements in an unconstrained pointing task, to see if characteristic deficiencies in the performance of Alzheimer’s disease (AD) patients could be identified. Groups of young controls, elderly controls, and patients with clinically probable AD were asked to point to small or large light targets, that were presented randomly in one of 4 locations, and that were illuminated either for 500 ms or until the target screen was contacted. For some blocks of trials the subjects were asked to point “as quickly as you can” while for others, they were asked to point “as accurately as you can.” All groups showed the same pattern of changes in their motor behaviour in response to the trial-by-trial changes in target characteristics. Although both control groups demonstrated changes in peak velocity, duration of deceleration, and accuracy with changes in the instructions, the AD group failed to modify their performance in accordance with the changes in the objectives of the task. This study illustrates that impairments analogous to those revealed on cognitive neuropsychological tasks may be evident in the motor planning and execution of relatively simple reaching tasks.

490.9
SYNCHRONIZATION AMONG MOTONEURONE POOLS DURING DIFFERENT PHASES OF CAT REACHING. M. L. McCurdy*, T. M. Hamm, and K. M. Horn. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85015.
Coherence spectra reveal the strength of synchronization between common neuronal systems or nerves and are used as evidence of common synaptic input (Christakos et al., 1991; Farmer et al., 1993). Coherence analysis of electromyographic records collected from elbow, wrist and digit muscles during a reaching task from one cat was used to determine synchronization during reach, grasp, hold and stance phases. Significant coherence was observed at lower (\textless{}20Hz) and higher frequencies. Stronger coherence at higher frequencies was present between proximal and distal muscles during the reach phase than during the grasp phase. Coherence at high frequencies was also found in comparisons among digit muscles or digit and wrist muscles during reach, grasp and hold phases, but not the stance phase. These findings suggest that there are common inputs to motor pools involved in the reaching task. In addition, inputs may differ during various phases of movement. Direct descending projections from the red nucleus to digit pools (McCurdy et al., 1987) may account for coherence among distal limb pools while interneuronal pools projecting magnocellular and parvocellular nuclei may be important during the reach phase. Supported by NS22454 and NS30013.

490.6
THE EFFECTS OF GRADED TACTILE ANESTHESIA ON THE CONTROL OF GRIP FORCE. K.J. Cole* The University of Iowa, Iowa City, IA 52242.
Tactile sensory information from the hand appears to provide information about discrete mechanical events during manipulation of objects (Hohmann, R.S. In Humphrey, D.R., Freund, H.F. Motor Control: concepts and issues. Wiley, New York, pp. 351-355, 1991). One prediction from this theory is that altered fingertip forces are unlikely at mild levels of tactile anesthesia. A sufficient population of afferents should remain to encode the necessary mechanical information. Tactile anesthesia of the hand was induced by variably compressing the median nerve at the palm in three subjects. Median nerve function was monitored by recording the compound sensory nerve action potential (SNAP) at the finger from electrical stimulation of the nerve at the wrist. The forces at the fingertips (normal and tangential, corresponding to the grip and lift forces) were recorded as subjects lifted an object using the thumb and index finger. Reductions in SNAP amplitude of 20-30% yielded small but significant decreases in tactile sensitivity. Fingertip forces were normal in their size and timing in all subjects. Substantial increases in grip force occurred when SNAP amplitudes were reduced by 50% or more. No subject could detect cotton stroked on the fingertips and all reported that their hand felt ’thick’. Grip forces returned to precompression levels when SNAP amplitudes returned to 70% of normal. These observations are consistent with the theory of discrete event sensory-driven control of grasp.

490.8
Valid measurements of spasticity are needed to evaluate effectiveness of therapeutic interventions. The angle at which EMG onset to passive muscle lengthening (threshold angle) occurs has been suggested as a measure of spasticity. However the conditions under which consistency in threshold angle responses within subjects occur remains to be determined. This study determined the effect of starting joint position and velocity of stretch (provided by a torque motor) on threshold angle within and between subject. Two starting angles and 2 stretch velocities (the conditions) were randomly assigned at each of 15 shortening sessions among 5 patients with spastic hypertonia. Threshold angle was determined from biceps and brachioradialis EMG responses. Starting angles used: 70° and 90°. Neither starting angle nor duration produced a significant effect on threshold angle (p<.05). A 90° starting angle and a 1.0 rad/s velocity stretch yielded the most consistent threshold angles between sessions within subjects. If data from these subjects can be extended to more patients with spastic hypertonia, then ability to use threshold angle to measure spasticity consistently should depend on: comparison within individuals, use of a consistent starting angle, and use of condition 90° and 1.0 rad/s across sessions. Supported by NIH Grant No. NS28784.

490.10
IS ACCURACY IN POINTING INFLUENCED BY DISTURBANCES TO POSTURAL KINESIOLOGY?: S. Prentice and I.S. Fink*. Dept of Kinesiology, Univ of Waterloo, Waterloo, Ontario, Canada, N3L 3G1.
Vibration of the posterior muscular systems is known to cause illusions of falling forward. In a previous illusion study we demonstrated one’s ability to locate a target in space: the arm’s position became elevated in the presence of Achilles tendon vibration. This study further investigates the relationship between postural kinesthesia and the performance of a goal-directed arm movement. Subjects (N=7) stood in the dark and pointed to a 5x5 matrix of diodes. Only those diodes located in the vertical midline were analysed. Bilateral vibration of the Achilles tendon was initiated after the target diode had been extinguished. In the previous study, vibration was present during target illumination. All movements were initiated with the arm at the subject’s side, and targets were located 20° and 5° above and below a shoulder angle of 90°. Trials were randomized with respect to target diode and vibration, with each combination being presented only once. Joint angles (Opatokon) and center of pressure data confirmed that subjects tended to lean backwards in response to the illusion of falling forward, which was induced by the vibration. The trunk orientation remained unchanged in the presence of vibration. Vibration also had no effect on the final position of the arm or on the accuracy of the pointing movement. The accuracy of pointing could be attributed to the effects of vibration on the perceived target location rather than postural orientation. Since vibration was not on during target illumination, there was not a false impression of target location and an accurate pointing movement was achieved. Thus, it appears that postural kinesthesia has a limited contribution to pointing movements when gravitational cues are present, and perception of target location dictates the pointing movement.
490.11


Previous work in our lab has shown a predictable pattern of ground reaction forces (GRFs), center of pressure (CP) and postural muscle activity (EMG) during visually cued fast-paced focal reaching movements in normal subjects. Following stroke, GRFs were prolonged and decreased in amplitude, CP excursions were both decreased and abnormal and postural muscle activation was delayed and had increased variance. In contrast to the literature, we found that patterns of muscle activation were variable and subject-specific in stroke. Moreover, muscle activation patterns were not related to pre-established clinical postural measures, but appeared to relate to the site of lesion (cortical (C) versus basal ganglia (BG)). We postulated that C lesions would have decreased reaction time/movement time ratios (RTMTs) compared to normals indicative of disordered movement execution whereas BG lesions would have increased RTMTs indicative of disordered movement preparation.

Current studies show that RTMTs are 14% lower for the C's and 11% higher for the BG's in comparison to controls. C's are unable to activate contralateral distal postural muscles whereas BG's are able to activate bilateral postural muscles but with inappropriate temporal sequencing. These findings support lesion specific sensorimotor processing deficits in postural regulation associated with goal-directed movements. Subject specific 1-D lesion reconstructions using MRI and differences in postural muscle activation strategies are presented for subjects with C and BG lesions during both cued and self-initiated fast reaching movements. (Supported by NSRDP R856606-1457-59)

490.12


Changes in control of aiming movements as a function of practice have been characterized in healthy, young adults (e.g., Proteau & Cournoyer, 1990), but the literature is silent about practice effects in the performance of these movements in adults with brain damage. Subjects with unilateral cerebral lesions have demonstrated differences in performance and control of aiming movements dependent on the side of the lesion. We suggested that each hemisphere has a specialized role in motor control (Haaland et al., 1987). The purpose of this study was to investigate the differential roles of the hemispheres in the control of aiming movements in adults with unilateral stroke. Two groups of right-handed adults with unilateral stroke (right-RS and left-LS) practiced a reciprocal aiming task in the same 500 target hits in each condition. Compared with the LS group, the RS group required more trials due to a higher error rate. Movement time group differences were largest early in practice. Kinematic analyses revealed differences in control strategies over practice. Overall, subjects increased horizontal velocity, especially in the less difficult condition. Using the vertical dimension revealed group differences which increased over practice. In contrast to the persistent low vertical target impact of the LS group, over practice the RS group appeared to use impact to minimize reversal time. The inability of the LS subjects to optimize the reversal despite practice is consistent with the role of the left hemisphere in the sequencing of actions (Winston & Pohl, 1994). To address the effect of handedness, healthy controls were tested and their performance was compared to that of these post-stroke patients. (Support: Foundation for Physical Therapy and the California Physical Therapy Fund)

490.13


We investigated the effects of surface texture (satin and sandpaper) on perceived weight in a task in which subjects lifted a reference object and then a test object with the same hand using a precision grip with the tips of the thumb and index finger at the sides. Subjects judged objects covered in satin to be heavier than objects of equal weight covered in sandpaper. The results suggest that the effect of texture on perceived weight may be due to grip force: the greater the grip force required to lift the more slippery satin objects is greater than that required to lift the sandpaper objects. These results suggest that the effect of texture on perceived weight may be due to grip force: the greater the grip force, the heavier the perceived weight. This conclusion was supported by a control study in which subjects grasped objects with the index finger and thumb above and below the object so that different grip forces for the two textures would not be required. In this case, there was no effect of texture on perceived weight. The implications of these results with respect to sensorimotor memory are considered.

490.14

AUTOMATIC ADJUSTMENTS OF REACHING MOVEMENTS TO A TARGET THAT MOVES UNPREDICTABLY. B. Di G. & P. Lyon, MRC HMBU, Institute of Neurology, Queen Square, London WC1N 3BG, UK.

We have investigated the limb adjustments that occur when reaching for a target that moves and how these responses are modified by the subject's intent. Subjects (n=8) reached out to touch a disc of light projected on to a screen in front of them. In a third of the trials, approximately 25ms after movement onset, the disc moved either to the left or the right. Subjects were required either to continue trying to point to the disc (P+) or to point in the opposite direction as soon as they perceived it to move (P-). This was contrasted with a reaction task in which subjects had to reach for their index finger stationary in front of them. When the disc moved, they had to either follow it (R+) or move in the opposite direction (R-). The position of the finger tip in space was recorded at 5ms intervals (Selspot).

In all conditions, when the disc moved, the finger tip began to move in the same direction approximately 130ms later. However, in the P- and R- conditions, this was followed by a second response in the opposite direction, at approximately 200ms. The magnitude of the early response was measured by calculating the lateral deviation of the finger during the period 130-190ms and finding the difference between target left and target right trials. These magnitudes were (mean ± SEM): P+ 18.1±4.4mm; P- 9.3±2.5mm; R+ 2.3±0.8mm; R- 7.0±2.9mm. The results show that arm movements can be adjusted at short latency by a moving visual stimulus. The fact that the early response was not suppressible by voluntary intent may suggest that it is an automatic or reflex component. The early response was much larger during a reaching movement than in the reaction task which indicates that the mechanism is task-dependent.

490.15


Multijoint arm movements of individuals with Huntington's Disease (HD) were examined using 3D kinematic analysis. Six HD subjects with moderate chorea and 4 age-matched healthy subjects performed rapid pointing movements to 1" target located at 3 distances, 2 of which required trunk motion. HD subjects were divided into high and low functioning groups (HF and LF) based on the Barthel index. Movement times of HD subjects were longer than healthy subjects (p<.005). All HD subjects had similar curvilinear hand paths, which were not reflective of an inability to move shoulder and elbow joints simultaneously. However, LF subjects showed greater variability in the hand paths and shoulder/elbow coordination, particularly in the fastest target which required substantial trunk movement. Whereas the movements of healthy subjects showed unimodal hand tangential velocity profiles, the movements of HD subjects were multimodal. For the HF subjects, a large initial movement was followed by smaller submovements (SM's) as the subject honed in on the target. Moreover, the peak velocity and amplitude of the initial movement scaled with target distance. The velocity profiles of the LF subjects were more variable, especially for the farther distance, and there was a larger number of SM's than the HF subjects. Additionally, the LF subjects did not scale peak velocity to displacement. Presence of SM's in all HD subjects could be due to increased force modulation, or to factors such as slowing of movement and greater accuracy constraints, as previously observed in healthy subjects (Müller & Iba, Neuroscience, 1990).

490.16

ADAPTATION TO CORIOLIS FORCE DISTORTIONS OF REACHING MOVEMENTS IN THE BLIND. J.R. Lackner*, R. Eastover, E. Bentzen, D. DiZio* Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254 and Psychology Department and School of Education, Boston College, Chestnut Hill, MA 02167.

Forward reaching movements during passive, counterclockwise body rotation (10rpm) generate Coriolis forces that deviate the arm to the right. As a movement slows down the Coriolis force abates and the arm trajectory bends back toward the intended path but still ends significantly to the right of pre-rotation endpoints. Normal subjects have straight line trajectories and accurate endpoints, without tactile feedback about target location, within ~20 reaches in darkness and 6 with sight of the arm. We exposed five congenitally blind subjects to Coriolis forces as they attempted to reach forward along their perceived body midline (distance, 32cm; peak velocity, 620mm/s). Relative to pre-rotation their movement trajectories were deviated rightward 55°, peak, and ended 47mm right. After 20 reaches their endpoints were again accurate and movement trajectories straight. Thus, blind subjects adapted to Coriolis force perturbations of their arm movements as well as sighted subjects despite vision. Vision is clearly not essential for motor adaptation to altered inertial characteristics of arm movements. Braschel proprioceptors, cutaneous receptors and effrent commands must be providing the information used to tune arm trajectory and endpoint. Supported by NAG 9-515 and NTS 6933002.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
In a reaction time task with two possible directions and two possible extents in each direction, direction and extent cues were considered equivalent in information-theoretic terms. Precise information about direction should have the same effect on reaction time (RT) as information about extent. Eight subjects performed a wrist pronation-supination RT task to one of two possible extents in each direction. One, two or four targets were lit prior to the GO signal to give five different conditions: (i) a single target lit specifying direction and extent; two targets lit specifying (d) direction only, or (ii) a choice of two direction-extent combinations; and (iv) four targets lit.

Subjects performed 320 trials per condition in four sessions of four blocks (100 trials each) randomly mixed recorded on different days. Results showed that direction prewcing was associated with significantly greater RT benefit (shortening) than extent. Prexuing extent but not direction was no more beneficial than giving a choice of two different yet completely specified targets, but did offer an advantage compared to conditions in which no precise information was available. These results indicate an equivalence in the way that information about direction and extent are represented in the brain. They are consistent with a model of motor programming in which movements involving antagonist groups of muscles are represented by different cell assemblies while level of activation within an assembly encodes movement extent.

**CIRCUITRY AND PATTERN GENERATION II**

491.2


A major action of second messenger systems is the modulation of the electrical properties of neurons. The effects can be complex, however. A single second messenger can directly modulate several types of ionic currents in a cell and these currents may indirectly affect other currents via voltage-dependent or Ca**+-dependent mechanisms. Thus, in order to understand these global actions of modulatory agents, it is necessary to have a detailed understanding of all of the relevant currents and their modulatory influences. One neuron which meets these criteria is the endogenously bursting neuron R15 in *Aplysia*.

We have implemented proposed mechanisms for the modulation of two ionic currents (Na and Ca**) associated with a model of R15. The model is sufficient to simulate a wide range of endogenous behavior in the presence of various concentrations of serotonin (5-HT) or dopamine (DA). Additional insights into the consequences of modulation of multiple currents were obtained by placing the model into a silent or bursting mode by various methods and examining the model’s response to extrinsic stimuli. Some of these insights were obtained by examining the Ca**+-dependency of I-V plots. Also, by selectively “clamping” specific variables within the model we were able to elucidate mechanisms through which depolarizing and hyperpolarizing pulses can terminate bursting and elicit a sustained (9-10 sec) postinhibitory hyperpolarization. The results suggest that the actions of modulatory agents and second messengers cannot be understood on the basis of their direct effects alone. It is also necessary to take into account their indirect effects on other unmodulated ion channels which occur through the dynamic interactions of voltage-dependent and Ca**+-dependent processes.
491.3 SEROTONIN REGULATES THE ELIGIBILITY FOR MULTIMODAL ACTIVITY IN THE BURSTING NEURON R15 IN Aplysia H. A. Lechler, D. A. Baxter, J. W. Clark and J. H. Byrne* Dept. of Neurobiology and Anatomy, and Dept. of Electrical and Computer Engineering, Rice University, Houston, TX 77005, and Dept. of Electrical and Computer Engineering, Rice University, Houston, TX 77251-1892.

Simulations of a mathematical model of the bursting neuron R15 in Aplysia, revealed that multiple stable modes of oscillatory electrical activity can coexist in this neuron when the stimulus is applied. The transient synaptic inputs can induce a persistent shift from one mode to another and that modulatory transmitters can regulate multimodal activity (Canavier, 1993).

Intracellular recordings from R15 revealed that long-lasting changes in synaptic activity can be induced by brief (0.3 - 1.5 s) depolarizations (5 - 10 nA) that trigger transient spikes (10 - 20 mV) caused by changes in the membrane input resistance, as predicted by the model. Under certain conditions such perturbations were able to switch the electrical activity of R15 between bursting and plateauing. The bursting persisted for varying durations ranging from ~30 sec seconds up to ~40 min. The effect of perturbations was dependent on two factors, steady-state bias current and the presence of serotonin (5-HT). Perturbations were ineffective for bias currents of <0.5 nA, whereas 30 - 40 % of the perturbations triggered transitions from bursting to plateauing with bias currents >1 nA. In the presence of 5-HT (2.5mM), multistability was observed at lower levels of bias current. With a bias current of 0.5 nA transitions to plateauing were triggered in 60 % of the cases, and with bias currents >1 nA 40 - 50 % of the perturbations induced transition.

We conclude that both 5-HT and depolarizing bias currents can regulate the eligibility of R15 for mode shifts, and thus may alter its sensitivity to synaptic input.

491.4 DETERMINATION OF SYNAPTIC POLARITIES USING A COMPUTATIONAL CELLULAR MODEL OF THE NEMATODE TAP PEPTIDE RECEPTOR ANTIBODY RECEPTOR CIRCUITRY OF THE NEUROPHYSIOLOGICAL Basis of Synaptic Transmission in C. elegans. C. J. Rockstein, A. S. Wicks and J. C. Rankin* Dept. of Computer Science and Engineering School of Medicine, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4.

The nematode C. elegans is a small soil-dwelling worm which exhibits many behaviours found in higher vertebrates, such as motor patterns, learning, and memory. Its genetics, neuroanatomy, and physiology are well understood to the point where it is possible to construct a model of a complete behaviour that includes all individual neuron and synapse.

We have previously constructed a computational model of the C. elegans tap peptide receptor system. The model consists of 7 sensory cells and 9 pairs of interneurons, which were found to play a role in the tap withdrawal reflex (Wicks and Rockstein, 1992), together with a "gateway" that models the motoneuron circuitry and mechanisms which trigger the movement evoked by the tap. Based on the evidence from the CPG, we have shown that a non-synaptic, sequential model of the neuron membrane potential and a two state, and graded model of synaptic transmission. Synaptic efficacy was inferred from anatomical data (White et al., 1986).

The polarities of the synapses are as yet unknown and we have developed a strategy to use our model to predict these polarities. We computed the polarities of all synapses using a constrained optimization based on the results of the model's predictions for the avoidance behaviour of the larval worm when different neurons were ablated using laser microsurgery. In addition, we used a modified functional test to determine interaction effects between connection polarities and other physiological parameters that describe membrane properties and synaptic activation kinetics.

491.5 EVIDENCE THAT SEROTONERGIC/NEUROMODULATORY INTRINSIC TO THE TRITONIA SWIM CPG IS DUE TO PRESEMBLY ENHANCEMENT OF RELEASE. P. S. Katz and W. N. Frost, Department of Neurobiology & Anatomy, University of Texas Medical School, Houston, TX 77030.

One of the neurons that controls the pattern generator (CPG) for escape swimming in the mollusc Tritonia doliolus evokes neuromodulatory effects upon other members of the CPG. We previously reported that stimulation of one dorsal swim interneuron (DSI) produced graded synaptic potentials evoked by neuron C2 onto two followers: the dorsal flexion neurons (DFN) and other DSIs (Katz et al., Nature 367:799-791, 1994). Our evidence now suggests that this neuromodulatory effect is mediated by serotonin acting on the presynaptic neuron (C2) rather than on its followers. 1) DSI enhanced the chemical synapses (both excitatory and inhibitory) of C2 onto each of its followers which have been tested, including the ventral swim interneurons (VSI), DSI, DFN, and pedal cells not directly postsynaptic to DSI. 2) DSI evoked little or no change in R15, recorded in the somata of C2 followers. 3) DSI-evoked modulation did not affect the ERS of the synapses. 4) If the effect were postsynaptic then inputs from other neurons onto the follower might be enhanced, yet DSI stimulation did not enhance the synapses from other DSIs onto the same C2 follower, but instead caused them to decrease.

Although the mechanism producing the presynaptic enhancement is not known, possible mechanisms are suggested by the following observations. Stimulation of the serotonergic DSIs decreased the hyperpolarization (APH) following C2 spikes. Likewise, exogenous serotonin also caused the C2 AHP to decrease. The decrement in AHP may be related to presynaptic currents Involved in transmitter release. Preliminary results indicate that DSI stimulation also may interact with the homosynaptic facilitation of transmitter release from C2. (This work was supported by NIH grant MH49635).

491.7 CACLIUM PLATEAUS AND GRADED SYNAPTIC TRANSMISSION IN LEECH HEART INTERNEURONS. G. O. Olsen, O. Nadim, and R. L. Calabrese, Dept. of Biology, Emory University, Atlanta, GA 30322.

Pairs of reciprocally inhibitory neurons in the leech CNS form the oscillator that controls the frequency of the heart rhythm. These pairs oscillate in antiphase, generating bursts with underlying plateaus and both graded and spike-mediated postsynaptic transmission. To find the extent to which low-threshold Ca currents contribute to plateaus and determine the duty cycle of the graded synaptic transmission, we voltage-clamped such a neuron with a voltage waveform that was obtained by removing the spikes from real waveforms. Its postsynaptic cell was voltage-clamped at -35 mV and showed the graded postsynaptic current associated with these presynaptic oscillations. The same experiments were also performed in a detailed conductance-based model of the two cells. The results confirmed the predictions of the model; the rise of the plateau cannot be completely accounted for by the Ca currents only. Thus other inward currents like the persistent N-type Ca current and the hyperpolarization-activated inward current (h) contribute in the process of building the plateau. The duty cycle of the graded synaptic transmission proved to be smaller than 0.5, thus suggesting that the spike-mediated transmission is necessary to produce the observed prolonged inhibition.

These results are supported by further experiments in Na-free saline where the persistent Na current and h-current were reintroduced through dynamic-clamp (Sharp et al., TINS 16: 369, 1993). To obtain non-synaptic oscillations, it was necessary to add these currents, thus demonstrating their role in maintaining the plateau. The plateau duration is shorter than that observed in normal saline where the graded synaptic transmission is supplemented with spike-mediated transmission.

491.8 MODULATION OF OSCILLATIONS IN A MODEL OF LEECH HEART INTERNEURONS. E. Nadim, O. H. Olsen and R. L. Calabrese, Dept. of Biology, Emory University, Atlanta, GA 30322.

The bursting oscillations underlying the heartbeat of the medicinal leech are generated by pairs of reciprocally inhibitory heart interneurons (HH cells). We have built a conductance-based Hodgkin-Huxley type model of a pair of HH cells using experimentally measured ionic and synaptic currents. The synaptic current in the HH cells has a graded component, which, in our model, is dependent on presynaptic Ca++, as well as a spike-mediated component. Our laboratory has previously shown that FMRFamide has excitatory effects on the HH cells, by changing the shape and amplitude of spike-mediated IPSPs, and by shifting steady-state activation and inactivation curves of the K+ currents. The effects of calcium antagonist were also shown to affect the IPSPs. In the model, in modulating the period of oscillations is similar to the modulatory effects observed in the biological cells. We are in the process of analyzing the effect of shifting the activation curves of the K+ currents in the model.

Even though the model cells are capable of oscillation merely with the graded component of synaptic transmission, the model cells are capable of oscillation merely with the graded component of synaptic transmission. This suggests that spike-mediated transmission can play a dominant local role in a network of neurons that has large graded synaptic currents. The dominant role of spike-mediated transmission is significant in light of the fact that neuromodulation of the heartbeat cycle rate is directed towards spike-mediated ipsp's and towards k+ currents that affect spiking.
491.9

491.10
INTERACTIONS BETWEEN SEGMENTAL LEG CENTRAL PATTERN GENERATORS IN ISOLATED LOCUST THORACIC NERVE CORDS. Y. Rybeckbusch* and C. Laurensen, Neuroscience Division, Computation and Neural Systems Program, Mail Code 138-74, California Institute of Technology, Pasadena, CA 91125.

Rhythmic motor patterns can be induced in leg motor neurons of isolated thoracic ganglia of the locust, Schistocerca gregaria, by a bath application of the muscarinic agonist pilocarpine (Rybeckbusch and Laurensen, 1994). We have recorded the rhythmic motor patterns evoked in preparations of thoracic ganglia in which the connectives between ganglia remained intact. Cross correlation analysis of the activities of different motor pools in the three thoracic ganglia revealed that the rhythmic motor patterns in different ganglia are centrally co-ordinated. In particular, thoracal le-

491.11
EFFECTS OF TEMPERATURE ON PROPERTIES OF LOCUST FLIGHT NEURONS: IMPLICATIONS FOR MOTOR PATTERN GENERATION. H. Xie* and R. M. Robertson, Dept. of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Elevated temperature causes a mild increase in wing-beat frequency of flying loci. This is mainly due to the global effects of temperature on neuronal properties of flight neurons in the CNS. The present investigation of flight neurons showed that an increase in conduction velocity (V0.5 = 1.53) and a decrease of membrane time constant (τm = 0.62) were the major factors which account for the increase of the CPG output. Hyperpolarization of the resting membrane potential (V0.5 = 1.8) and reduction in input resistance (τm = 0.54) might be involved in automatic compensation for the effects of increased temperature. Also increases in temperature above room temperature decreased the amplitude of postsynaptic potentials (PSPs).

We have investigated the change of resting membrane potential and the PSP amplitude contributed to the observed temperature effects on the frequency of the central rhythm. The temperature dependent hyperpolarization of flight neurons was mimicked by changing the K+ concentration of the saline from 10 mM to 2 mM. After 5 min high K+ superfusion, the resting membrane potential of flight motorneurons hyperpolarized from an average of -39.1 nV to -47.8 nV, while the frequency of the central rhythm decreased from an average of 10.1 Hz to 8.8 Hz. The temperature-dependent change of PSPs recorded from flight neurons was mimicked by replacing normal saline with zero Ca++/high Mg++/ saline. Although the amplitude of PSPs was more than halved after 10 min zero Ca++/high Mg++/ treatment, the frequency of the central rhythm changed minimally (<1 Hz). These results support the hypothesis that resting membrane potential directly affects the central rhythm, whereas synaptic strength could serve simply to trigger endogenous mechanisms of burst generation.

491.12
QUANTIFICATION OF S-RHYTHM IN NEURAL POPULATIONS BY COHERENCE ANALYSIS: C.N. Christopoulos*, Med. School, Univ. of Crete, Greece, and Ch. New York, Columbia University, U.S.A.

A recent study (C.N. Christopoulos Neurosci. 58, 43-57, 1994) indicated that for a neural population comprising uncorrelated units and units that are correlated around some frequency Fs, the unit-to-aggregate (UTA) coherence function is near zero for the uncorrelated subset but has a nonzero value at Fs for members of the correlated subset. This value reflects the inhomogeneous size of the population and the characteristics of synchrony (extent and strengths of unitary correlations, and phase concentration for the correlated units); and as indicated by simulations of a uniform population, it is substantial for wide ranges of values of these parameters. Thus, UTA coherence computations on a sample of units allow the estimation of the extent of population synchrony, as well as indications on correlation strengths, i.e., a quantification of neural synchrony. Current mathematical analysis using the same model of synchrony verifies these results for the UTA coherence. It further reveals that the value of the aggregate-to-aggregate (ATA) coherence at Fs between unit (sub)populations reflects the same four parameters, and is also substantial in a wide range of conditions. However, from this value the characteristics of synchrony cannot be separately estimated. Therefore, the ATA coherence at Fs only provides a general measure of synchrony, on the condition that the sizes of the two (sub)populations stay relatively fixed. Finally, the analysis shows that the UTA and particularly the ATA coherence are very sensitive indicators of population synchrony, especially when synchrony is weak (unit-to-unit correlations < 0.3).

491.13
P H SENSITIVITY OF SPONTANEOUS NETWORK ACTIVITY OF MAMMALIAN NEURAL NOS. S. R. Lauder* and G. W. Gross, Dept. of Biological Sciences, University of North Texas, Denton, TX 76203.

The association of pH changes with normal and pathological neural activity, as well as the influence of pH on major ligand-gated ion channels, has been established in several cell culture systems and in animal models. However, pH effects on the activity of small networks in well controlled culture environments have received less attention. We investigated the effects of pH, close to and within the physiological range, on the asynchronous activity of a small network of entorhinal mouse spinal neurons. Neurons were cultured on multielectrode plates to permit simultaneous extracellular monitoring of network activity. The pH was shifted by altering the CO2 flow in bicarbonate buffered medium in the recording chamber and was continuously monitored. Spike data was stored on 14 channel analog tapes for later analysis, and a chart recorder displayed the raw data. The results indicate that spontaneous activity is highly sensitive to pH over the range of 7.0 to 8.0. Using activity at pH 7.3 as reference, total spike production, as reflected by cell firing rate, increased as low as 1% at pH 7.2 and increased as much as 66% at pH 7.7. With increasing pH, burst frequencies increased as did the appearance of large complex bursts. This pattern reversed as pH was lowered, with large bursts disappearing below pH 7.2. Under bicarbonate dissection (producing coordinated, regular bursting) a burst decrease from 7.4 to 7.2 was observed both burst durations and frequency decreased to 40%. Burst frequencies increase from 7.4 to 7.7 caused burst frequency decreases increased to 30%, but duration increases of up to 40%. This degree of pH sensitivity necessitates that quantitative similarity between commonly performed tests and controlled pH environments. In addition, pH may be a useful variable in determining burst initiation and burst maintenance mechanisms.

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481.15

THE USE OF SYNAPTIC POTENTIALS AS THE CHARGE ON PARTICLES IN THE GRAVITATIONAL REPRESENTATION OF NEURONAL ASSEMBLIES: RESULTS FROM SIMULATIONS


The gravitational representation of simultaneously recorded spike trains (Genethin et al. 1985) provides a conceptual framework for the analysis of the dynamics of neuronal assemblies (Lindsey et al. 1982). Each neuron is represented as a particle carrying a time varying charge that is a filtered version of spike activity. The charge is calculated on each neuron; the charge on a neuron at each instant of time is the time integral of the difference between the neuron's spike times and the firing times of a reference neuron. The charge on each neuron is a measure of the deviation of the actual firing times of the neuron from the firing times of the reference neuron. The gravitational representation of neuronal assemblies is a measure of the deviation of the actual firing times of the neuronal assembly from the firing times of a reference neuronal assembly.

481.16

GRAPHICAL MEASURES OF SPIKE TRAIN SIMILARITY AND COUPLING BASED ON RECURRENCE TIME HISTORY MATCHING (RTHM).

B.K. Rhoderick, J.M. Kugelki, J.F. MacKay and C.W. Gross, Dept. of Biological Sciences and Physics, Univ. of North Dakota, Grand Forks, ND.

We have applied the Hausdorff metric and related similarity functions as cumulative and running time estimators of similarity or coupling between two spike trains, based on the local auto-recurrence times associated with each spike. These new methods have been tested with biological spike trains from cultured mouse spinal cord networks, as well as artificial spike trains from a class of stochastic models. The central algorithm operates on two spike trains and a particular cross pair of spikes A and B, and begins by finding the best match for each of N sequential auto-recurrence times of spikes of A in the time history of spikes of B. This constitutes a set of N minimal A->B distances. A set of N minimal B->A distances is similarly determined. The maximal distance (DAB) from the A->B and B->A is sums is a sensitive measure of local spike train coupling. By comparing DAB to a predetermined threshold θ, one can find the set of associated L values corresponding to cross pairs of spikes with criterion matches in each of the two spike trains. The RTHM is also appropriate as a running-time measure of coupling laminar macro and nano, for detecting coupling between microspatial data sets. By graphically combining this similarity function with the rank mean local inter spike interval we have produced a clear demonstration that networks can become functionally decoupled during parietal bursts, as a manifestation of activity-dependent gain. Supported by the State of Texas Advanced Technology Program.

481.17

A NEW HIGHER-ORDER INTERSPIKE INTERVAL ANALYSIS FOR DETECTING SEQUENTIAL FIRING TRENDS IN NEURONS.

D.C. Tam,* Center for Network Neuroscience, Dept. of Biological Sciences, University of North Texas, Denton, TX 76203.

A new spike train analysis method has been developed to detect how transitions of firing intervals in neurons are dependent on higher-order interneuronal intervals (ISIs). A set of "firing trend indices" is introduced to detect trends in firing independent of time scales. The firing trend index (k) is defined as the ratio between the first-order ISI and the second-order ISI relative to a reference spike. Since the value of h is bounded by 0 and 1, (i.e., 0 ≤ h ≤ 1) counting how many times firing intervals are changed may be made independent of the absolute time scale of the underlying intervals. As the value of HI increases, 0 represents the existence of change from short to long ISIs, and vice versa, for hI → 1 and hI → 0, respectively.

In addition, another firing trend index k is defined as the ratio of the difference between the two adjacent ISIs to the second-order ISI, k = hI - hI+1, where h = 0 indicating no change in firing intervals, k → 1 indicating extreme lengthening in ISI, and k → -1 indicating extreme shortening in ISI. Plotting these indices against the sequential spike number provides a clear indication of the evolution of normal firing trends in the brain. Thus, the transition into and out of burst firing, for instance, can be detected easily. Furthermore, plotting these indices against the first-order ISI will provide an estimate of the transitional probability of spike firing. Using these new state transition analyses, the underlying state transition processes, such as Markov processes or other processes, may be revealed in neurons. (Supported by ONR N00014-93-1-0135)

481.18

A FUZZY CLUSTERING APPROACH TO THE RECOGNITION OF MULTINEURON ACTIVITY.

G. Zouzidaki* and D.C. Tam,* Dept. of Electrical & Computer Engineering, University of Houston, Houston, TX 77204-4793 and Center for Network Neuroscience, Dept. of Biological Sciences, University of North Texas, Denton, TX 76203.

Typically, spike discriminative techniques can be used to extract separate spike trains from many neurons contributing to a recorded signal, and to create reliable spike templates for each neuron.

For this purpose, we use a fuzzy clustering approach to extract multi-unit activity. In our procedure employs fuzzy K-means clustering on a data segment containing multi-unit activity (1) to identify the number of neurons contributing to the recorded signal, and (2) to create reliable spike templates for each neuron. For this purpose, a performance index is also used as an optimization criterion.

Comparison of performance between the conventional crisp K-means and our fuzzy K-means clustering approaches is done with synthetic spike trains generated using real spikes and segments of background noise recorded at high rates. Both conventional and fuzzy clustering simulations clearly demonstrate the advantage of the fuzzy procedure in identifying the exact number of neurons and the exact templates over a wide range of signal-to-noise ratios and several "fuzziness indices". They also allow us to determine the optimum "amount of fuzziness" for real-time processing.

This method can be applied to other electrophysiological signal analysis, such as EEG and evoked potentials. (Supported by ONR N00014-93-1-0135)

481.19

LEVEL OF ACTIVITY AND INHIBITION EFFECTS IN A SPINAL MOTOR CIRCUIT SIMULATION.

D.P. Bashor* and D.M. McDermott, Dept. of Biology, University of North Carolina at Charlotte, NC 28223.

A computer simulation based on R.J. MacGregor's (1987) SYSTM series was used to investigate the importance of level of network activity on excitation and inhibition in a spinal motor circuit. The spatial distribution of Renshaw cell (Ren) excitation by Mn was also studied. Single Mn and eMn populations (169 cells each) were interconnected with 5 pairs of interneuron populations in a network composed of about 2300 cells. The network was driven by 5 pairs of fibers representing IA and IB motor pools, and tonic descending excitation. At low levels of network background firing, Mn activity was quenched immediately after termination of input. At high levels of background, after effects made only small differences in number and number of active Mn cells, as well as providing little reciprocal inhibition of the antagonist population. The effects of focal activation of Ren by Mn compared with widespread activation demonstrated complex effects with the network. Changing from widespread to focal activation of Mn by MnMs had little effect on Mn activity, but a rather larger effect on eMn frequency. However, both mean frequency and number of cells active changed in Ren and eRen populations.
All of the muscles had diverged force fields (because the force never reversed direction. It should be mechanically impossible for a muscle to produce a stable force field). There were three primary directions in which those divergent force fields were pointed. Radially away from the body (Pyridinum, Quadratus Femur, Peroneus, Vastus Externus, etc.), the Femoral blood vessels (Semimembranosus, Adductor Magnus, Rectus Femoris [major and minor], and toward Biceps, Bicep, Pectoralis, Rectus Anus, Adductor Longus, Gastrocnemius, and Sartorius). Thus, there is redundancy in the muscle force fields. In addition, some of the muscles had zero-valued force fields throughout some part of the workspace. The muscles Rectus Anus, Rectus Femoris [major and minor], Gastrocnemius, Adductor Longus, Vastus Externus, and Sartorius all showed a complete or near complete loss of force throughout some portion of the workspace. The utility of this type of muscle force field may lie in its ability to provide position-dependent force field modulation without requiring position (afferent)-dependent EMG modulation. Because none of the muscle force fields were stable, we conclude that limb stability can only be achieved through appropriate combinations of muscle activations. A primary function of the spinal cord may be to assure stability by serving as a focus for implementing the co-ordinated muscles.

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492.2


Doubles, or two repetitive stimuli delivered with a short interpulse interval, have been shown to produce a force output that is greater than the linear sum of force produced from one stimulus (Burke et al. Science 1970). In addition, this effect may be motor unit or muscle fiber type specific. We previously showed that the human chronically paralysed soleus muscle uncharacteristic of physical responses to the stimulus, while the acutely paralysed soleus muscle (less than 6 weeks) responds as a slow muscle. This study examined the effects of the repetitive doublet on the human chronically acutely paralysed soleus muscle evoked twitch after and before a standard fatiguing regime.

Ten chronic (one year) and two acute (6 weeks) completely paralysed individuals had their leg positioned in a torque measuring device with the knee at 90° of flexion. The soleus muscle in vivo was monitored during all stimulations to ensure supramaximal activation. Three interpulse intervals (6 msec, 12 msec, 18 msec) for the doublet were delivered in six possible combinations via the visual naive. Each combination was preceded by a single twitch. Next, a fatigue protocol consisting of a 20 Hz frequency delivered every second for 330 msec was delivered for two minutes. This protocol lead to significant fatigue. Immediately after the fatigue protocol the same doublet protocol was administered. Dependent measures included peak force, force-time integral, and the rate of force production both before and after the fatigue protocol. Following the fatigue protocol, the doublet lead to approximately 3 times the peak force and force-time integral than that of a single stimulus in the chronically paralysed soleus muscle. Conversely, the acutely paralysed soleus muscle showed no significant change in the output from the doublet. This study suggests that increased force production from the both muscles is muscle fatigue dependent. Mechanisms and implications of these findings will be discussed.

492.3


Spinal isolation (SI, transection of the spinal cord at T2-T13 and at L5-S1) and deafferentation between the two transection sites) was produced to inactivate the cat soleus for 4 months. We examined the effects of cyclic activity (1 Hz) mimicking the step cycle with electrical stimulation (40 Hz, 300ms, 30mV) during either lengthening (SI-L) or shortening (SI-S) on the (functional deficits associated with chronic inactivation: MWL, muscle weight, TPT, isometric twitch time to peak tension, Po, maximum isotonic tension, Vmax, maximum rate of shortening; FL, fatigue index) (% difference from control.)

<table>
<thead>
<tr>
<th>Group</th>
<th>MWW</th>
<th>Po</th>
<th>TPT</th>
<th>Vmax</th>
<th>FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI-L</td>
<td>64</td>
<td>83</td>
<td>15</td>
<td>90</td>
<td>1</td>
</tr>
<tr>
<td>SI-S</td>
<td>45</td>
<td>68</td>
<td>41</td>
<td>29</td>
<td>12</td>
</tr>
<tr>
<td>S-L</td>
<td>47</td>
<td>60</td>
<td>38</td>
<td>58</td>
<td>5</td>
</tr>
</tbody>
</table>

The force frequency curve was shifted to the right of a faster type muscle after SI. This effect was accentuated by programmed activity. In the vivo soleus tendon was shortened by 9% and 9 times more active than passive manipulation in the SI-L and SI-S cats, respectively. SI-L resulted in a faster and flatter muscle with general daily programmed activity ameliorating the adaptations in MW, Po, and FL. SI-L had a greater effect than SI-S in Po and FL. The relative N0fatigability of the soleus was maintained in all groups. Thus, as little as 30 min/day of activity has a positive effect in maintaining the mechanical properties of a chronically inactivated muscle.

Further, stimulation during lengthening was more effective than shortening probably reflecting the faster force integrals during lengthening. (Supported by NIH Grant NS16333 and NRSA HD 7416).

492.4

COMPARISON OF FORCE-FREQUENCY RELATIONS IN HUMAN VOLUNTARY AND STIMULATED CONTRACTIONS. L.J. Tremblay, G. Roy*, B. Bigland-Ritchie, Dept. of Pediatrics, Yale University School of Medicine, New Haven, CT. 06510 and The School of Physical and Occupational Therapy, McGill University, Montreal, Canada.

In voluntary contractions muscle force is modulated by a combination of motor unit recruitment and rate-coding. When either whole muscle or single motor units are stimulated at different rates, a sigmoidal relationship is observed. These results, in contrast, during voluntary contractions, mean spike frequencies increase linearly with force up to 100% MVC (Bigland-Ritchie, et al. 1991; Thomas et al. 1991); a force which usually matches that for supramaximal stimulation. The purpose of this study was to compare, in the same muscle, forces generated by electrical stimulation with those generated by motor units recorded during voluntary contractions of various intensities.

Muscle force and motor unit firing rates were recorded from the quadriceps muscles of 16 subjects during voluntary contractions varying from 25% to 100% MVC. In separate experiments, quadriceps muscles were stimulated electrically at rates from 1 to 100 Hz. The two force-frequency relationships were compared. For forces up to about 60% of MVC, there was little discrepancy between voluntary and stimulated excitation rates. However, for higher forces the stimulated force increments per Hz became progressively smaller, while this non-linearity was largely avoided during voluntary contractions. Possible explanations for this discrepancy include: 1) the progressive recruitment of larger units at higher forces; 2) that forces from different units add non-linearly when their territories overlap (Clamann & Scholtz, 1988); and 3) whether or not the CNS can excite all units to respond at maximal rates. Supported by NIH grants NS14755 & HL02062, PCAR (Quebec) & NSERC (Canada).

492.5

DOUBLET POTENTIATION IS DIMINISHED DURING SHORTENING MOVEMENTS OF CAT SOLEUS MUSCLE. J.G. Sanderson, C.C. Henneman. Department of Physiology, Northwestern University School of Medicine, Chicago, IL 60611.

When motor units are stimulated with low frequency trains, the addition of a single extra pulse, or doublet, at the beginning of the train can dramatically increase tension during an isometric contraction (Burke et al, Science 158: 1287-1289, 1967). This study investigated the effects of muscle movement, using either isovelocity ramps or simulated locomotor patterns, on doublet potentiation in whole muscle or single motor units in cat soleus. The light line in Fig. A shows the isometric force produced by stimulating a portion of the soleus with a 10 Hz train. The heavy line depicts the addition of a doublet (pulse added 10ms after the first pulse). In B, using identical stimulus patterns, the same muscle was shortened at 20mm/sec from 0.2 to 0.7a, and then allowed to contract isometrically. Shortening movements diminished the effects of the doublets in whole muscle and motor units. Less dramatic results were seen with slower or shorter isovelocity ramps, or with locomotor type movements. Moderate isovelocity stretches of similar speed and distance had little effect on doublet potentiation.

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492.7

MODELING ISOMETRIC FES MUSCLE FATIGUE BY ARTIFICIAL NEURAL NETWORKS. M. Domany, Y. Yadin, Pecht, J. Mizrahi, E. Isakov and Z. Susuk. Dept of Biomechanical Eng., Technion, Haifa 32000, Israel and Loewenstein Rehabilitation Hospital, Ra'anana, Israel.

This study investigates the relation between the input electrical current applied to the paralyzed human biceps brachii muscle in vivo during Functional Electrical Stimulation (FES) and the output muscle force. The electric current is applied through surface electrodes to the muscle, and the force is measured in isometric conditions. The assumption made is that the muscle during FES consists of the following two parameters: 1. Muscle length, described by the knee angle θ (°); 2. Current frequency, f (Hz). 3: Current pulse width, D (0.25 ms each) and 4: Current amplitude, A (0 to 130 mA). The output is muscle tension T (N). T at time t is the function of the input history to the muscle. We model T using a sampling rate of 1 Hz to represent the current amplitude A. The output force is very non-linear and it is difficult to estimate its magnitude in advance. We use 2-layer feedforward artificial neural networks simulations to learn the forward dynamics model of the force produced in the muscle. The training stage is done by supervised learning with the backpropagation algorithm. Following the training stage with muscle fatigue data of A = 50 or 70 mA FES, the neural network predicts the output force for the unknown case of muscle fatigue induced by 60 mA FES. The network can also extrapolate the output muscle force during a 50-60 seconds period, after learning the forward dynamics model of muscle force production during the first 50 seconds of FES stimulation. We propose that by using appropriate neural network architectures one can represent knowledge of FES experiments by training the weights of the networks. The network weights could be eventually stored on VLSI chips and help in the design of appropriate controllers aimed to replace the motor brain in activating paralyzed muscles. This study was supported by the Segal Foundation and the Sandra and Walter Kaye fund.

492.8

ELBOW-JOINT IMMobilIZATION DECREASES FATIGABILITY AND ALTERS THE PATTERN OF ACTIVATION IN HUMANS. G. Yau*, M. Hildago, and R. M. Enoka. Dept. Biomedical Engineering, The Cleveland Clinic Foundation, Cleveland, OH 44195

The left arm of 8 subjects was immobilized in a fiber-glass cast and a sling for 4 weeks at an elbow joint angle of 90 degrees. Twenty-four hour chronic EMG recordings indicated that the activity of the biceps brachii muscle was significantly reduced during immobilization. Before and after immobilization, the following variables were measured: (1) elbow-flexion force, and EMG signals from the biceps brachii and brachioradialis muscles during maximal voluntary contractions (MVCs); (2) fatigueability of the elbow-flexor muscles when force was sustained at 20% of the MVC force; (3) cross-sectional areas and volume of the biceps and triceps brachii, and brachioradialis muscles measured with magnetic resonance imaging (MRI); and (4) the degree of muscle (biceps brachii and brachioradialis) activation during an elbow-flexion MVC as evaluated with MRI T2 relaxation time. The immobilization induced a significant decrease in the elbow-flexion MVC force and cross-sectional areas and volume of the elbow flexor muscles, but the reduction in muscle size was less than the decline in MVC force. After immobilization, the fatigueability of the elbow flexors was decreased (more resistant to fatigue) at both force levels (20 and 65% MVC). There were also significant changes in the EMG activity and T2 indicating degree of activation during the MVC. The neuromuscular adaptations were diverse and had different effects on the various physiological processes. Supported by NIH grant NS 20544 (RME).

492.9


Following either supramaximal electrical stimulation or maximal voluntary contractions sufficient to induce fatigue, a full or near full recovery of the muscle compound action potential (M-wave) has been observed within 3 minutes of cessation of the fatiguing process. (Galea and McComas, J. Physiol. 438:212T, 1991). Little attention has been given to the excitability during recovery, and we have found evidence of a late depression.

Fatigue was induced by 20 Hz supramaximal stimulation of the motor nerve of the biceps brachii muscle in healthy men of varying activity levels, and recovery period was examined for up to 8 hours post fatigue. Immediately after cessation of the fatiguing stimulus the amplitude and area of the M-wave had returned to control values whereas the isometric twitch force was significantly reduced in all subjects. The M-waves declined throughout the recovery period, with one group (n=9) reaching a maximum M-wave depression (DEP) between 7 to 30 minutes post fatigue (60.4 ± 9.0% control) whereas another group showed maximum M-wave depression much later in the recovery period, between 13 minutes and 4 hours post fatigue (DEP* = 42.4 ± 6.2 % control). The isometric twitch force recovered gradually, returning to control values by the end of the 8 hour recovery period. The depression of M-wave activity may be explained by a reduction in Na-K pump activity or by increased open times of the sodium channels.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS IX

493.1


This model, utilizing coupled mechanisms for place recognition, maintenance of head direction, and path integration, replicates a variety of rodent behavioral and neurophysiological data. We simulated experiments by Collett, Cartwright, and Smith on gerbils driven to a field open landmark-based maze. Experiments by Cheng on rats navigating to a goal location in a rectangular arena. Experiments by Munsie and Muhlnad in which the gerbils possess a clear and accurate sense of head direction. Head direction cells have been found in several areas of the rat brain.

In addition to a path integrator and units coding for head direction, our model contains units with place fields controlled by visual landmarks, reflecting properties of hippocampal pyramidal cells. However, unlike other models of place cells, our model also accounts for the persistence of place fields in the absence of any visual input, by allowing place units to be driven by the path integrator. This, in our model, is learned to form associations between external state (views of landmarks) and internal state (path integrator coordinates). Visual cues, when available, drive the place units, which can then correct for drift in the path integrator, suggesting it is a known value when the animal is first placed in a familiar apparatus, such as a radial maze.

Our model shows how allocentric bearings derived from head direction can be used to disambiguate visually identical landmarks, reproducing the gerbil and rat data. Conversely, local view information can be used to correct for drift in the head direction estimate. Parallel alignment among place units produces a consistent place code even when the model is presented with a distorted landmark array, emulating the robustness that animals show in novel situations. Parts of the model have been implemented on a mobile robot.

493.2

AROUSAL DEPENDENT ACTIVATION OF FD MOLECULAR LAYER GLYCOCEN PHOSPHORYLASe-A. A.Ucker*, G. Rao, B.L. McNaughton, C.A. Barnes, T.A. Foley and F.M. Reiman, ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

"Patches" of glycogen phosphorylase-a activity observed in the fascia dentata (FD) molecular layer (Harley & O'Keefe, 1992; Wallace, 1982) occur following microinjections of glutamate into the entorhinal cortex (Harley & O'Keefe, 1986) and are correlated with the depletion of glycogen stores in the nervous system during metabolic work (e.g. Wallace, 1983). In addition, reduced phosphorylase-a activity in the FD molecular layer after exploration (Ucker et al, 1994) concurs with single cell recording studies (Ranck, 1973; Thompson & Best, 1989) showing that most hippocampal neurons are least active during behaviors typically associated with hippocampal theta rhythm, i.e. active exploration and paradoxical sleep. Place cell activity is maintained when an animal explores or is manually transported to an identified place field, but drastically reduced after it has been snugly restrained (Poster, et al., Science, 244:1580, 1989), although the theta rhythm is only marginally reduced. We hypothesized that phosphorylase activity would be minimized under restraint, relative to animals either freely exploring, manually moved, or anesthetized with sodium pentobarbital. FD molecular layer glycerogen phosphorylase-a activity was measured in 12, 6 month old female Long-Evans rats. Contrary to the firing-rate based expectation, phosphorylase-a activity was least during exploration, higher during passive movement, higher yet during restrained movement, and highest under anesthesia. The mitobol F-ratio was significant (p < .05). Phosphorylase-a expression may thus be more correlated (inversely) with hippocampal theta rhythm and/or septohippocampal activity than with principal cell firing-rates per se. Supported by MH48024, MH30897 and the Flinn Foundation.

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493.3 TOWARDS A COMPUTATIONAL MODEL OF THE HIPPOCAMPAL FORMATION INCORPORATING REALISTIC ANATOMICAL CONNECIVITY: AMMON’S HORN AREA CA3, P. Patton and B.L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

A computational model of the rat hippocampal formation incorporating realistic anatomical connectivity is being developed. This report focuses on Ammon’s horn (CA1, CA2, CA3, and DG). Neurons were distributed throughout a rectangular sheet representing this structure. 250,000 pyramidal cells were assumed (West et al., Anat. Rec. 231:482-497). Major extrinsic inputs to CA3 derive from the entorhinal cortex and FD granule cells. Entorhinal input possesses a rough topographic organization, and is divergent, with individual arbors extending up to 2 mm septotemporally (Tannamura and Noyol, Hippocampus, 347-370). Entorhinal arbors were represented as rectangles extending across CA3. Each entorhinal neuron makes about 4700 synaptic contacts onto pyramidal cells (Amaral et al., Prog. Brain Res. 83:1-11). Each granule cell makes about 15 mossy fiber contacts onto pyramidal cells (Claiborne et al., JCN 246:435-458). CA3 pyramidal cells possess a network of recurrent collaterals, represented in the model as diagonal sawths like those described by Ishizuka et al. (JCN 295:580-623), with each cell receiving 6,000 ipsilateral synaptic contacts. These cells also provide a significant input to the inner molecular layer of FD (Li et al., JCN 339:181-200). 5 groups of CA3 non-principal cells were represented: vertical and horizontal basket cells, chandelier cells, spiny non-principal cells, stratum oriens cells, and superficial cells. CA3 contains an estimated 67,000 non-principal cells (Dics et al., Dev. Brain. Res. 81:885-894). This anatomical representation will serve as a basis for models of postulated hippocampal operations such as associative memory. Supported by MH46823.

493.4 SIMULATION OF THE SPONTANEOUS REACTIVATION OF EXPERIENCE-SPECIFIC HIPPOCAMPAL NEURONS DURING SLEEP. B. Shen and B.L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

During slow-wave sleep (SW) in both rats and monkeys, periods of spatial activity, CA1 "place-cells" that were temporally correlated, by virtue of the overlap of their place fields, exhibit enhanced temporal correlations, even though the animals sleep in a different location. Recording rats were displaced from their home cages (for 2 days before testing) and subjected to "daylight" conditions (14L:10D). The discharge of cells with overlapped place fields was more correlated in subsequent sleep, particularly during sharp-waves, than in sleep episodes generated by concurrent behavioral patterns. These results are consistent with the hypothesis that the hippocampus is involved in the consolidation of memories. The results support the view that the reactivation of neuronal assemblies may be an important mechanism for the consolidation of memory, and that the hippocampus plays a role in this consolidation.

493.5 SPARSE VS. DISTRIBUTED POPULATION CODING IN SUPERFICIAL AND DEEP LAYERS OF RAT NEOCORTEX. W.-E. Skaggs*, B.L. McNaughton, G. Winson, K. Moore, and C. Duffield. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

324 cells were recorded extracellularly from two areas of the rat neocortex (Zilles’s areas HL and OC2m), using a parallel recording method that permitted monitoring of up to 40 individual neurons at the same time. We have been using a parallel recording method (Winson and McNaughton, 1992), in which neurons are assumed to be in a state of random to be silent for extended periods of time. Overall the population of activity appears much more sparse in the superficial neocortical layers than in the deep layers. These findings are of interest in light of the fact that the superficial layers are the predominant source of the cortico-cortical association system. In memory, our proposal has been for this system by theorists including Marr (1970) and Eccles (1978, et al); and it has been shown mathematically that sparse activity patterns confer special benefits on associative memory networks by reducing interference between jointly stored patterns. Moreover, the majority of neocortical NMDA receptors are located in the superficial layers (Monaghan and Cotman, 1989). Supported by MH46823 and MH00897.

493.6 POPULATION ACTIVITY PATTERNS IN HIPPOCAMPUS AND NEOCORTEX PERSIST LONGER DURING SLOW REM SLEEP THAN DURING SLOW-WAVE SLEEP. K.M. Moore, W.E. Skaggs, B.L. McNaughton, C.A. Barnes*, R. D’Monte, C. Duffield. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

To gain insight into the possible role of sleep in memory consolidation, we quantified the structure of population dynamics during different behavioral states. Using a parallel recording method (Winson and McNaughton, 1992), populations of up to 40 neocortical cells or up to 70 hippocampal cells were recorded concurrently in the neocortex or in the hippocampus of rats, during behavioral states of REM sleep, slow wave sleep (SWS), or performance of a spatial task. The persistence of population activity patterns in each state was quantified by calculating the correlations between the firing rate vectors at different times, with care to exclude high-rate cells (putative interneurons) from the populations. In each behavioral state, the mean correlations decayed more or less exponentially as a function of time interval, and the time constant of decay was taken as a measure of the average persistence of an activity pattern. The approximate time constants observed, both in neocortex and hippocampus, were SWS, 100-1500 ms; REM sleep, 5-10 sec; active locomotion, 400-1000 sec. As might be expected, the persistence of correlated states during behavior appears to be a function of the behavior itself. During SWS, correlations persist on a timescale similar to hippocampal-slow-wave activity consistent with a possible hippocampal origin of the correlated states. During REM, the persistence is not correlated with any obvious neocortical or hippocampal EEG phenomena. These findings add to the extensive evidence for similarities between REM sleep and the waking state. Supported by MH46823 and MH00897.

493.7 PRESERVATION OF TEMPORAL ORDER IN HIPPOCAMPAL MEMORY REACTIVATION DURING SLOW WAVE SLEEP. M.A. Wilson* and B.L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

In a previous study, it was found that coactivity of cells during behavior produced an increase in discharge correlation between these cells during subsequent slow wave sleep (Wilson and McNaughton, 1994). This suggested that hippocampal memories for recent experience were recapitulated during these slow sleep periods. The question which remained unanswered was whether the temporal structure of experience was also preserved and was present during sleep activity. To address this question, 50-100 cells per area CA1 of the hippocampus were recorded simultaneously during two 20 min periods. Individual pairwise correlation histograms taken during these tasks were classified based on their direction of skew. In each pair, if firing of cell 1 (master cell) was greater than the firing of cell 2 (slave cell), this pair was assigned a positive skew. The converse relationship was assigned a negative skew.

During behavior, this skew resulted from the relative spatial locations of the "place fields" of the cells, with the specific patterns that would follow during each task. It was found that the direction of skew was positively correlated with behavior (peak shift 1-2 sec) determined during the direction of skew of the immediately following slow wave sleep period (peak shift 5-10 sec). This indicates that the structure and temporally compressed order of recently experienced states are recapitulated in the hippocampus during slow wave sleep. The preservation of both coordinated states and their temporal order suggests a mechanism within the hippocampus which is capable of storage and reactivation of temporally sequenced information. Supported by MH46823 and McDonnell-Pew.

493.8 VARIATIONS IN PLACE-SPECIFIC FIRING OF HIPPOCAMPAL NEURONS ALONG THE SEPTOTEMPORAL AXIS. M.W. June*, S. Wiener, M. Moore, S. Kipen, and B.L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

The anatomical and neurochemical organization of hippocampal circuitry exhibits a substantial septotemporal gradient. Although it is well established that place-specific firing is a fundamental characteristic of hippocampal neurons, almost all unit recording studies in freely behaving animals have come from the temporal lobe, and involves technical difficulties. In our study, therefore, single units were recorded in the dorsal and ventral hippocampus of opposite hemispheres in the same rats, in many cases simultaneously. Units were presumed to be identified as rats entered slow wave sleep. The rats then performed a food reinforced, random search task in a square chamber containing simple visual landmarks. As in dorsal hippocampus, ventral CA1 could be classified as "complex spike" (CS)-pyramidal cells or "theta" interneurons. Both dorsal and ventral theta cells fired at relatively high rates with low spatial selectivity in the area. As previously reported (see June et al., 1996) and as expected behavior of the animal was highly correlated among the CS cells in the ventral hippocampus; however, significantly fewer ventral units had "place fields" than dorsal units, and the spatial selectivity of ventral units was significantly lower than that of dorsal units. Thus a septotemporal gradient of spatial selectivity was found in the CA1 field of the hippocampus, complementing many other anatomical and neuropharmacological studies. A number of possible interpretations can be suggested from these results, including a computational advantage of representing space at different scales or a precedence of essentially non-spatial information processing in the ventral hippocampus. Supported by NS02031 and Human Frontiers in Science.
HIPPOCAMPAL PLACE FIELDS CHANGE WITH NAVIGATIONAL CONTEXT. Y. Qin, E.L. Markus, B.L. McNaughton and C.A. Barnes. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

When general behavioral requirements are held constant, rat hippocampal complex spike cells fire selectively with respect to the animal’s location. The locations of ‘place fields’ of individual cells are generally quite robust against manipulations of the animal’s environment and are relatively unaffected by age-related changes, with some exceptions. When firing rates are modulated by contextual variables such as running velocity and direction, the location of firing is relatively unaffected by the specific behavior. In the present study, the effects of the navigational requirements of the task were assessed in fixed environments. Hippocampal place cells were examined in adult male rats aged 30 to 45 days and were trained to forage for chocolate either on a radial arm maze or a circular platform. The rats would first retrieve randomly scattered chocolate; then the task was altered by placing the food sequentially and repeatedly in 4 specific locations. This resulted in a switch from a random to a directed search pattern by the rats, typically over the course of 1-2 minutes. On this platform, it also involved a change in the trajectories and distribution of locations visited. The switch from random to directed search was accompanied by a pronounced change in the location of the place field in approximately 1/3 of the cells on the circular platform. There was a smaller, but significant effect when the recordings were conducted on the radial arm maze. In 2 rats the directed search was typically followed by a second random search episode. In these cases, the place fields returned to their original location. We conclude that, if hippocampal cells encode location per se, the representation changes in different behavioral or navigational contexts. Supported by MH00897 & ONR.

DYNAMICS OF VISUAL CUE CONTROL OVER HEAD DIRECTION CELLS AND PLACE CELLS. L.J. Krieter*, H.S. Kortnev and B.L. McNaughton. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Thalamic head direction (HD) cells and hippocampal place cells comprise a tightly coupled system. We investigated the dynamics of visual cue control over these cells by introducing conflicting direction information between visual cues and the animal’s head direction. Rats foraged for food in a high-walled test apparatus with a single salient cue card. In some experiments, we disoriented the rats before each session. This weakens visual cue control over place and HD cells (Krieter et al., Soc. Neri, Abt., 1993), because most HD cells had preferred firing direction or location relative to the cue card over multiple sessions. In 30% of 119 sessions, however, firing orientation rotated 30°-130° within the session. A similar effect was observed in the first few runs for rats from an “incorrect” orientation to the “correct” one. In 6 cases, cells rotated from one incorrect orientation to another. In 3 cases, cells started at the correct orientation, rotated away, but then returned. In no cases did cells permanently rotate away from the correct orientation. In other experiments, we abruptly rotated both apparatus and rat by 180°. Typically, cells either immediately lost orientation with the cue, or remained oriented relative to the external world. In other sessions, however, cells initially maintained their external orientations, but then rotated 180° smoothly over a few min until they returned to the original orientation relative to the cue card. Thus, visual cues can correct for large errors in spatial orientation with a slow, steady rotation of both the HD cell and the hippocampal spatial map until they realign with the cue. The progressive shift suggests that transitions between neighboring network states are preferred over transitions among less similar states. Supported by ONR and NS09052.

AGE-RELATED DECREASE IN THE NMDA-MEDIATED EPSP IN FD. G. Sagg, C.A. Barnes, B.L. McNaughton and I. Lavenstein. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Previously we demonstrated a decline in both the non-NMDA and NMDA-mediated Schaffer collateral EPSP in senescence CA1. Here, we characterize the NMDA response in senescent fascia dentata (FD), where, although there is a smaller presynaptic fiber potential for a given stimulus intensity, indicating a decrease in perforant path fibers, the non-NMDA EPSP is actually larger for a given fiber volley amplitude (Barnes and McNaughton, 1980), indicating that individual perforant path synapses of old animals are more powerful (Lavenstein et al., 1991). P64 rats (n=18, 9 mo; n=18, 24-28 mo) showed an age-related deficit in spatial learning ability in the spatial version of the Morris water task, (% in time to largest quadrant: yng = 45.3 ± 5.2; adult = 37.1 ± 7.4; p<0.05). There was also an age-related deficit in spatial discrimination ability. Hippocampal slices were then prepared and incubated in low Mg+4 ACSF. Recording and stimulating electrodes were placed in FD. Input-output curves of the relation between the presynaptic fiber volley and EPSP were constructed under and after local application of a non-NMDA (AMPA) antagonist. Abolition of the residual response by APs confirmed that it was NMDA-mediated. As found previously, prior to CNQX application the EPSP-to-fiber volley ratio was significantly larger in the old rats. After CNQX application, the NMDA component for a given fiber volley was unchanged. This indicates no impairment in visual spatial learning ability. Hippocampal slices were subsequently prepared, and the atropine-sensitive, cholinergic slow spew was recorded intracellularly in CA1 and CA3 pyramidal cells. There was an age-related deficit in spatial learning ability. Hippocampal slices were then prepared, and the atropine-sensitive, cholinergic slow spew was recorded intracellularly in CA1 and CA3 pyramidal cells. There was an age-related deficit in spatial learning ability. There were no age differences in intrinsic biophysical properties of principal cells within each subgroup; however, the amplitude of the cholinergic slow spew was significantly reduced in CA1 (45% CA3-55%) and CA3 (55-65%), p<0.001. The 3 wk and 9 mo rats did not differ. The slow wave in CA1 was correlated with spatial learning on the Morris water task in old rats more significantly (p<0.05) in the older relative to the younger age groups. This result demonstrates that components of excitatory synaptic transmission within the same hippocampal subregion are differentially affected by the aging process, even when the receptor subtypes may be colocalized. Supported by AG03576 and MH00897.

AGE-RELATED DECREASE IN CHOLINERGIC SYNAPTIC TRANSMISSION IN FD. H.A. Chen*, G. Yao and C.A. Barnes. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

The electrophysiological changes of neurotransmitter cholinergic deficits in normal aging has been examined in area CA1 of hippocampus where the cholinergic slow spew has been found to be reduced in aged rats (Petier et al., 1991; Taylor and Griffith, 1993). Whether this change occurs in all three hippocampal subregions and whether it covaries with age-related behavioral deficits has been studied here. F-344 rats (n=3, 3 wk; n=3, 9 mo; n=30, 24-27 mo) were tested in the Morris water task. There was an age-related deficit in spatial learning ability. Hippocampal slices were subsequently prepared, and the atropine-sensitive, cholinergic slow spew was recorded intracellularly in CA1 and CA3 pyramidal cells. There was an age-related deficit in spatial learning ability. There were no age differences in intrinsic biophysical properties of principal cells within each subgroup; however, the amplitude of the cholinergic slow spew was significantly reduced in CA1 (45% CA3-55%) and CA3 (55-65%), p<0.001. The 3 wk and 9 mo rats did not differ. The slow wave in CA1 was correlated with spatial learning on the Morris water task in old rats more significantly (p<0.05) in the older relative to the younger age groups. This result demonstrates that components of excitatory synaptic transmission within the same hippocampal subregion are differentially affected by the aging process, even when the receptor subtypes may be colocalized. Supported by AG03576 and MH00897.
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493.15 ALTERED IEG INDUCTION AFTER LTP IN MEMORY-DEFICIENT AGED RATS. A. Lanahan, T.F. Whorley, G. Lyford, G. Stevenson, G. Rao, L. Church, and C.A. Barnes. ARL Division of Neuronal Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724 and Dept. of Neuroscience and Neurology, Johns Hopkins Univ., Baltimore, MD 21205.

Aged rats show deficits in the maintenance of hippocampal LTP and in spatial memory. To identify possible molecular mechanisms of these changes, we examined the regulation of a panel of immediate early genes induced in young and old LTP. Seven young (2-6 months) and 7 old (26-30 mo) F344 rats were bilaterally implanted for electrodes for evoking and monitoring perforant path field potentials in fascia dentata (FD). Recording commenced 2 days after surgery and was continued every 2 weeks after surgery; at which time rats were given 50 mg/kg cycloheximide (i.p.), followed by 1 hour of low-frequency test stimulation (0.1 Hz). Five young and 5 old rats received bilateral remote shocks of 0.1 ms duration at high-frequency stimulation every 5 min, and 2 rats in each age group were used as implanted controls. Poly (A)+ mRNA (Fasttrack, Invitrogen) was prepared from FD that was microdissected from the rest of the hippocampus, and used to prepare cDNA by reverse transcription (Superscript, BRL). The cDNAs were [32P] labeled by random priming and used to screen a panel of IEGs with the "Reverse Northern" technique. Hybridization to each of the IEGs was quantitated (phosphomager, Molecular Dynamics) and normalized to a non-inducible mRNA, glutathione phosphate dehydrogenase (GPD). Most IEGs were similarly induced in the young and aged animals however the induction of c-fos was much more robust in the old than the young animals and this difference was significant at p = 0.034. These observations indicate that mechanisms controlling the induction or turnover of c-fos are selectively altered in aged, behaviorally impaired animals. Support: AG02919, MH80997.

493.17 CEREBELLAR MECHANISMS OF EYELID CONDITIONING: MATHEMATIC ANALYSIS OF PLASTICITY AT TWO COMPETING SITES. G.T. Kerkut and M.B. Wisk, Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225.

Empirical evidence suggests the involvement of two sites of synaptic plasticity in single form of motor learning: one in the cerebellar cortex and another in the cerebellar nucleus. A mathematical model based on the synaptic organization of the cerebellar-dlivery system is used to analyze the relative contributions from these two sites. Based on experimental data, we assume that plasticity at synapses from granule to Purkinje (Gr-Pk) cells displays generalized long term depression: synaptic weights decrease when active during elevated calcium induced by climbing fiber input and increase when active in the absence of climbing fiber input (during which calcium levels should be reduced). We find that incorporating standard generalized Hebbian plasticity at synapses from mossy fibers to cerebellar nucleus cells (mf->nuc) leads to non-stationary stochastic weight strengths which are reduced when a generalized Hebbian rule is modified such that mf->nuc synaptic weights only decrease when active during tonic Purkinje cell inhibition, synaptic weights become stable. Furthermore, the system is self-regulating such that eventual activity is always driven to equilibrium levels at which synaptic weights remain constant. We next simulate idealized conditioning trials. Unconditioned stimulus, which increases the excitatory drive to climbing fibers, induces adaptive changes in the Gr-Pk and mf->nuc synaptic weights encoding the conditioned stimulus (CS). We assume that Gr-Pk synapses provide greater temporal discrimination such that a single subset of mf->nuc synapses but many different subsets of Gr-Pk synapses are activated throughout the CS. We show that each subset of Gr-Pk synapses active during the CS attempts to transfer plasticity to the nucleus. Thus, the relative contribution to conditioned responses made by plasticity at mf->nuc synapses is reduced to the weighted average across all subsets of Gr-Pk synapses active during the CS.


The amygdaloid neuronal model was constructed from single neuron activity data [1]. Significant features were the acquisition to conditioned stimulus and extinction after acquisition. A mathematical model of the amygdaloid neuronal network was presented to quantitatively produce the elemental physiological mechanisms of learning. General model for single neuron is proposed by McCulloch-Pitts model. Main features of the McCulloch-Pitts model are all-or-none response, potential summation and synaptic delay. Added features to model were the acquisition and extinction to conditioned stimulus. These features were described by learning weight between neurons. When the conditioned response is followed by the unconditioned response after short delay, connection weight increases. The connection weight becomes interneuronal. Details of temporal firing activities of the models were examined under various conditions. Results show that neurons acquired responses to conditioned stimulus associated with unconditioned ones and these responses were extinguished by extinction.


The NMDA receptor in the hippocampal CA1 area is suggested to play a role in memory function. Delayed Conditional Discrimination (DCD) tasks are used to investigate working memory in 7 and 7 old (26-30 mo) F344 rats were bilaterally implanted for electrodes for evoking and monitoring perforant path field potentials in fascia dentata (FD). Recording commenced 2 days after surgery and was continued every 2 weeks after surgery; at which time rats were given 50 mg/kg cycloheximide (i.p.), followed by 1 hour of low-frequency test stimulation (0.1 Hz). Five young and 5 old rats received bilateral remote shocks of 0.1 ms duration at high-frequency stimulation every 5 min, and 2 rats in each age group were used as implanted controls. Poly (A)+ mRNA (Fasttrack, Invitrogen) was prepared from FD that was microdissected from the rest of the hippocampus, and used to prepare cDNA by reverse transcription (Superscript, BRL). The cDNAs were [32P] labeled by random priming and used to screen a panel of IEGs with the "Reverse Northern" technique. Hybridization to each of the IEGs was quantitated (phosphomager, Molecular Dynamics) and normalized to a non-inducible mRNA, glutathione phosphate dehydrogenase (GPD). Most IEGs were similarly induced in the young and aged animals however the induction of c-fos was much more robust in the old than the young animals and this difference was significant at p = 0.034. These observations indicate that mechanisms controlling the induction or turnover of c-fos are selectively altered in aged, behaviorally impaired animals. Support: AG02919, MH80997.

493.18 CLASSICAL CONDITIONING AND VALUE-DEPENDENT LEARNING IN NOMAD, A REAL WORLD ARTIFACT. P.F. McBryde, V. Verschure, J. Wray and O. Edelman*. The Neurosciences Institute, 3777 North Torrey Pines Court, La Jolla, CA 92037.

In previous work, it was demonstrated how adaptive behavior can occur in a real world artifact, NOMAD, carrying out a tracking and a block-sorting task (1). NOMAD is a mobile automaton with visual and auditory sensors and a magnetic sensor that allows it to "taste" the environment of colored drums it picks three times. The behavior of NOMAD is controlled by a simulated neuron-based nervous system containing circuitry for the execution of reflexes and fixed or sensory-driven action patterns (avoidance, approach, exploration). States of the sensors are projected onto mapped and non-mapped neural areas. Initially, the value system is fixed and innate (I.e. 'light is better than no light'). It is modeled in terms of the properties of a diffuse ascending system that can modulate synaptic plasticity. Recently, the modeling of value has been expanded to include the experience-dependent modifications of value itself (acquired value) (2). In the present work, acquired value was incorporated into the nervous system of NOMAD. Our goal was to show how behavioral regularities resulting from classical conditioning can be understood in terms of value-dependent learning. A recent experiment conducted takes place in a neural region that is an analog of the amygdala. Acquired value is achieved by synaptic modifications of projection from the sensory area to the diffuse ascending system. As a result, discrimination of color preferences associated with value became more rapid and the responses became more marked. To test this anatomical and dynamical arrangement NOMAD is being subjected to classical conditioning paradigms emphasizing blocking and secondary conditioning with auditory stimuli. We analyze the conditioned behavior of NOMAD in terms of the success of avoidance and approach responses, the correlated states of its nervous system, and the effect of modifications in the stimulus environment. I. G. Edelman, et al., PNAS, 89, 7627-7632 (1992). K. J. Frisman, O. Tozawa, G. N. J. Reek, O. Sprem, G. M. Edelman, Neuroscience, 59, 220-243 (1994).

493.20 MODELING BIOLOGICAL NEURONS WITH SPATIOTEMPORAL EVENT MAPPING (STEM) CELLS S.B. Murphy* and E.W. Kaniels. Interdepartmental Neuroscience Program, Department of Psychology, Center for Theoretical and Applied Neuroscience (CTAN), Yale University, New Haven CT 06511.

A biological neuron can be viewed as a match-filter that instantiates a mapping, x, from multidimensional spatio-temporal (x,y,t) to unidimensional temporal events (action potentials). A computational abstraction of a biological neuron called a Spatio-Temporal Event Mapping (STEM) cell has been designed to perform this mapping in a general way. The three major components of the STEM architecture are (1) an input time-space mapping layer (2) a nonlinear spatial mapping layer (3) a recurrent time-space mapping layer. A STEM cell implementation of the McCulloch-Pitts model are all-or-none response, potential summation and synaptic delay. Added features to model were the acquisition and extinction to conditioned stimulus. These features were described by learning weight between neurons. When the conditioned response is followed by the unconditioned response after short delay, connection weight increases. The connection weight becomes interneuronal. Details of temporal firing activities of the models were examined under various conditions. Results show that neurons acquired responses to conditioned stimulus associated with unconditioned ones and these responses were extinguished by extinction.

493.21
SEQUENTIAL INFORMATION PROCESSING: THE NECESSITY FOR MAPPING TIME INTO SPACE. K. Schill, C. Zetzsche and E. Poppel. Inst. for Med. Psychology, Univ. of Munich, 80336 Munich, Germany

Problems in modeling the perception of dynamic scenes have casted doubts upon the concept of "iconic memory". Analysing current concepts of visual temporal information processing reveals that some basic inconsistencies regarding the temporal properties have not yet been resolved. We have developed a model for the representation of spatio-temporal information in early vision [1]. Its key feature is the mapping of temporal structure into simultaneously accessible locally distributed activities. Although the model is aimed at the description of spatio-temporal information processing, application to tectoscopically presentations also predicts basic results obtained in the field. Furthermore, the model resolves inconsistencies of processing rates obtained with partial report and backward masking paradigms. The standard view on neural information processing, that information is represented by the spatio-temporal activity of discrete elements (neurons), is shown to necessarily imply a discrete internal representation of time. We believe that the need for the proposed mapping is so fundamental as to be expected to appear in a distributed fashion on various parallel and hierarchical levels in the nervous system. However, since on subsequent levels, sequences with larger temporal extension have to be represented, information reduction has to be applied to avoid combinatorial explosion of connections. Future theories and investigations should concentrate on the syntactical and semantic aspects of perceptions and how they are represented. [1] Schill K., Zetzsche C. (1994): A model of visual spatio-temporal memory: the icon revisited. Psych. Res., in press.

493.22
INFORMATION TRANSFER IN NETWORKS WITH HEBBIAN CONNECTIONS. E. Salinas, E. Marder* and J.F. Abbott. Biology Department and Center for Complex Systems, Brandeis University, Waltham, MA 02254.

In many systems, firing rates of a population of neurons code for the value of an external variable. We investigate how information about the coded quantity can be communicated from one population (or topographic map) to another. We also explore how nervous systems might combine these representations in order to carry out coordinate transformations. These problems are analyzed using computer simulations the responses of cells that are broadly tuned to an external quantity. We have implemented methods to decode the information in such ensembles of neuronal activities (Salinas and Abbott, J. Comput. Neurosci., in press). This allows us to more explicitly examine the relationships between the established through a correlation based, or Hebbian type of learning mechanism. This corresponds, for example, to the generation of a motor command in the same direction as a given sensory input. Furthermore, the same algorithm can be used to couple two maps, so that the resulting set of activities codes for a linear combination of the original coded quantities. In this case a coordinate transformation is being carried out. These results demonstrate how networks can perform important computational tasks using biologically plausible learning mechanisms, such as the Hebb rule.

493.23

A neural network model of associative memory is proposed for information processing in the hippocampus. Recent morphological and physiological findings suggest a three-dimensional neural network structure in the hippocampus is considered for the modelling. We have adopted an assumption that each lamellar network accepts its respective category of information in this model, while the networks between granule cells and CA3 cells are constructed within each lamella, in which the information is processed according to their respective categories. The granule cells competitively select particular signals by negative self-feedback for each category. Further the recurrent paths in the CA3 cells strengthen the associative learning within a category. Both granule and CA3 cells fire sparsely. Association among different categories of information is made by the following inter-laminar connections of the CA1 and subiculum areas. This associative interaction has a general tendency to reduce excessive correlation among the signals. Therefore, sparse firing inputs to the CA1 cells are quite reasonable to protect from the excessive interference. The output direct input paths from the entorhinal cortex to these areas are very important. These looped circuits can lead the associated output signals to the appropriate input parts of the entorhinal cortex. We can understand the three-dimensional structure and the functions of the hippocampus by this model, and the importance of the hippocampus for associative memory as well.

493.24
NEURON EXCITABILITY AND ASSOCIATIVE MEMORY FUNCTION. E. Cook*, M. Migliore, and D. Johnston*. Div. of Neurosciences, Baylor College of Medicine, Houston, TX 77030 & Inst. of Interdiscip. Appl. of Physics, Natl. Res. Counc., Palermo, Italy.

In previous work we showed that the ability of three different biophysical models of a hippocampal pyramidal neuron to use synaptic potentiation as a mechanism of memory storage is dependent on a match between the magnitude of the synaptic input and cell excitability (Cook et al. Soc. Neurosci. Abs., 600.4, 1993). This previous work, however, only explored memory function in model neurons that were initially at rest and in a steady state. In the present report we have compared how previous synaptic activity reduces excitability of the three different neural models and tested whether this affects subsequent associative memory function.

Our three models were reconstructed hippocampal pyramidal neurons with the following different channel distributions and types: 1) passive dendrites with H&H-like Na+ and K+ channels in the soma, 2) passive dendrites with a full compliment of voltage-gated channels in the soma, and 3) a complete biophysical model that included active dendrites (Migliore et al. Soc. Neurosci. Abs., 295.13, 1993). Activation curves were computed for each model at various time points after four different stimulus paradigms: a) somatic current injection, and b) low (subthreshold), c) medium, and d) strong synaptic inputs. The program NEURON by Michael Hines was used in all computer simulations.

Comparisons between associative networks constructed from all models show that correct recall of stored patterns is affected by previous synaptic activity in models 2 and 3 but not 1. Simulations demonstrate that the two most realistic models (2 and 3) have increased thresholds to firing after experiencing all four types of stimulus paradigms. As synaptic input is increased (c and d), however, model 3 shows even greater reductions in excitability. This is a direct result of residual Ca2+ activating AHP K+ channels. Model 3, which contains dendritic Ca2+ channels, was affected the most by all three types of synaptic activity. (Kerr Center and MHE10475, MHE4754, MHE4832, and NS1535.)
494.1


The neural activity from parietal cortex (PG) to hippocampus (HF) is important for spatial recognition memory. In the present study, Sprague-Dawley rats were trained to perform a spatial working memory task. The rats were examined during the post-training delay period. Single unit activity was recorded from the PG and HF of an awake rat. While unit recording, the rat could be rotated in some direction, and the animal was directed to a match or a non-match (Fig. 1). There were 12 of 57 neurons in the PG and 10 of 30 neurons in the HF that responded to auditory directional stimuli. Ten neurons in the PG also had significant activity during the delay period that sometimes became enhanced after the rat was rotated to an unfamiliar direction. None of the HF-neurons responded during the delay period, but occasionally activity would emerge after the rat was rotated. These results suggest that spatial working memory in the PG is persistent, whereas that in the HF is transient.

494.3

RETROGRADE AMNESIA FOR SPATIAL DISCRIMINATION FOLLOWING ENTBROCHIAL CORTEX OR PARIAL CORTEX LESIONS IN RATS. Y. Chio, K. Nishio, T. Fujiki, K. Hashimoto, A. Fujikura, and H. Okada. Dept. of Psychology, University of Utah, Salt Lake City, 84112 Utah, USA; 2. Lab. Neurosciences, Compartements & cognitives, CNRS URA 339, Avenue des Facultes, 33405 Talamone Cedex, France.

Our previous study has demonstrated that lesions of entorhinal cortex (EC) produced a temporarily graded retrograde amnesia for spatial discrimination in rats. The present research was conducted to study further retrograde amnesia using rats as subjects as well as to extend to parietal cortex (PC), another brain area involved in the generation of spatial memory.

Rats learned successfully, one at a time, and every two weeks, 6 single-pair discriminations in two radial mazes (2 pairs in an 8-arm maze and 4 pairs in a 12-arm maze). Either the PC lesions or the EC lesions were made by unilateral aspiration of the PC or entorhinal cortex, respectively. The lesion operation was performed on the day after subjects had attained learning criterion on the 5th discrimination. Postoperative retention performance was assessed only for the last four discriminations indicated that the PC-lesions impaired overall performance as compared to control rats. The EC-lesions produced a more severe impairment than the PC lesions in addition to an extended temporal gradient of retrograde amnesia. The EC-lesioned rats were significantly impaired in retention of the 3 recent discriminations acquired immediately and up to 4 weeks prior to surgery, but were not different from controls for the first discrimination learned 6 weeks before surgery.

These results are consistent with previous findings obtained in mice with ibotenate lesions of the EC and suggest that the EC as well as the PC might be differentially involved in long-term storage/retention of spatial discrimination memory in rodents.

494.4


The rat medial prefrontal cortex (mPFC) consists of two distinct subregions, the prelimbic (PL) and the anterior cingulate cortex (ACC). Most behavioral investigations have treated these subregions as homogenous, therefore their individual contribution to the numerous cognitive functions ascribed to the mPFC remains unclear. The present study examined the effects of transient lidocaine-induced lesions of the PL or ACC on delayed spatial win-shift (DSWS) behavior on a radial-arm maze. The DSWS task consists of a training phase where 4 of 8 arms are baited randomly and the remaining 4 arms are blocked, and a test phase where all 8 arms are open but the previously blocked arms are baited.

Lidocaine micro-injections (2.5 -10 ul/group) into the PL or ACC produced no effect on: 1) training phase performance, 2) subsequent phase performance as assessed when the aesthetic effects of lidocaine had dissipated. Similar injections into the ACC had no effect on training phase performance, however lidocaine animals made significantly more entries into previously baited arms during the test phase. Transient lidocaine lesions of the PL delivered prior to the test phase disrupted test phase performance as lesioned animals foraged randomly. Similar microinjections to the ACC also impaired test phase performance, but in this case the errors were predominately revivals to previously baited arms. During the random foraging task in which 4 of 8 arms were baited transient lesions of the ACC were again accompanied by significantly more revivals to baited arms. PL lesions had no effect on this task. Given that PL lesions disrupted foraging for 4 pellets on the DSWS, but not during the random foraging task, the PL may play a role in complex rule-guided behaviors that utilize previously acquired information. Alternatively, the perseverative pattern of responding observed following transient lesions of the ACC suggest that the ACC is involved in situations which require newly acquired information to be used in a flexible manner.

494.6


We examined the effects of hippocampal and medial prefrontal cortex (MFP) lesions on discrimination of temporal duration and on memory for duration in rats. One short duration (2 sec) and one long duration (8 sec) was used and involved visual presentation of a 3-dimensional object. In the discrimination experiment, the door separating the rat from the stimulus was opened at the end of the time period, and latency to approach and move the stimulus was measured. For half of the rats, the stimulus covered a piece of food on the short duration presentations, but not the long durations, and the other half had the opposite contingency. In the second experiment, memory for duration was tested. Discrimination training (from 8-32 trials) took place for 2 weeks (i.e., 8 trials per day duration followed one second later by a test phase. The test phase involved presentation of one of two different 3-D stimuli. One object was always correct during both discrimination and retention phases. Latency to approach and move the stimulus was measured for both discrimination and retention phases. Memory for duration was tested. Rats in both groups received control, hippocampal, or MFP lesions following acquisition (significantly greater latencies on nonreinforced trials as compared to reinforced trials). Hippocampal and MFP lesioned groups showed performance deficits following surgery as compared to the control-operated groups on both tasks. Performance on the duration discrimination task returned to normal as compared to controls and pre-surgery performance, whereas both the hippocampal and MFP lesioned groups continued to perform at chance levels on the memory for duration task. Both the hippocampus and the medial prefrontal cortex appear to be involved in data-based memory for temporal information.

It has recently been shown that excitotoxic lesions of the anterior cingulate cortex facilitate early learning, and posterior lesions impair late learning, on a conditional visual discrimination task (Bussey et al., Soc. Neurosci. Abstr. 19: 1233, 1993). Furthermore, excitotoxic lesions of the ventral limb of the diagonal band of Broca (VDB), which result in cholinergic denervation of both these cortical areas, produce both of these effects on the same task (Muir et al., Soc. Neurosci. Abstr. 19: 1233, 1993). These results are concordant with those of others, who suggest that the anterior cingulate cortex is involved in mechanisms operative during the early stages of learning, while posterior cingulate activity predominates during later stages (Gabriel et al., Exp. Brain Res. 86: 585-600, 1991).

The aim of the present study was to determine whether the late-learning effects observed in our previous experiments were independent of the fact that animals were required to learn the task under the influence of the lesion. Accordingly, rats were trained to a criterion of 70% correct responding on two consecutive days, at which point animals received either quisqualate, acid lesion of the anterior or posterior cingulate cortex, or excitotoxic AMPA lesions of the VDB. Following recovery, the animals were returned to the task and trained until they had achieved 85% correct responding on two consecutive days. The results reveal that effects on late learning observed in our previous studies were indeed reproduced: both VDB and posterior cingulate lesioned animals showed an impairment in the ability to progress through the later stages of learning the task. In addition, the effects of the lesions on a post-acquisition retention test and on extinction were examined.


Cingulate cortex - the division of midline limbic cortex overlying the full rostrodorsal extent of the corpus callosum - has received little attention relative to other limbic structures such as the hippocampus. Recently it has become apparent that the cingular cortex can be anatomically dissociated into anterior and posterior components. However, the characteristic behavioural functions of these structures remain to be elucidated. Results obtained using an active avoidance paradigm suggested that the anterior cingulate cortex mediates mechanisms operative during early learning, while the posterior cingulate cortex is involved predominantly in late learning (Gabriel et al., Exp. Brain Res. 86: 585-600, 1991). More recently, it has been demonstrated that lesions of the anterior cingulate cortex facilitate early learning, and posterior cingulate cortex lesions produce a deficit in late learning, of a visual conditional discrimination task which requires the acquisition of a stimulus-response response (Bussey et al., Soc. Neurosci. Abstr. 19: 1233, 1993).

These results have led us to investigate the role of anterior and posterior cingulate cortices in the learning of stimulus-reward associations. We have recently developed a new computer automated touchscreen testing procedure which enables the testing of rats on various tasks involving the presentation of computer graphic stimuli. The present study employed this technique to examine the effects of anterior and posterior cingulate cortex lesions on simple visual discrimination and reversal and the acquisition of an 8-pair concurrent discrimination task. The results show that neither anterior nor posterior cingulate lesions affected acquisition of a simple discrimination. However, lesions of the anterior cingulate cortex impaired the early but not the late stages of acquisition of the 8-pair concurrent discrimination task.

494.11 INSULAR CORTEX GRAFTS RESTORE REMEMBERANCE OF PREVIOUSLY LEARNED TASTE AVERSIONS. C.E. Ormsby, V. Ramirez-Amaya and F. Bermudez-Rattoni* Instituto de Fisio! Celular, UNAM, Mexico, D.F., Mexico 04510.

Previous studies in our laboratory have shown that cortical grafts are able to induce rapid recovery of the ability to acquire a conditioned taste aversion (CTA) in insular cortex (IC) lesioned rats. Furthermore, tissue specificity has been shown since only autologous grafts induce recovery whereas occipital cortex (OC) tissue does not. Further studies have shown that IC lesions interfere with short and long term memory for CTA. The aim of the present study is to evaluate the effect of IC grafts on the recovery of memory aspects of CTA.

Twenty-week-old Wistar rats were trained for CTA by pairing a saccharin solution (0.1%) with a gastric malaise induced by an ip injection of LCI solution (0.15M). After 4 days to allow consolidation, all animals except a control (Ctrl) group were lesioned by microinjections of NMNOA and 10 days later the lesioned animals were randomly divided in 3 groups: one received IC grafts from 15-day-old fetuses (TwOC), one received OC grafts (TOWC), and one remained lesioned. Sixty days after grafting, all animals were tested for the CTA to saccharin and then retrained for a CTA to saline taste. The animals that remaining lesioned did not recall the saccharin CTA nor were able to acquire the new CTA to saline. Both grafted groups showed a recall of the CTA for saccharin similar to the control, but only the TwOC group showed values similar to the control for the acquisition of the new CTA for saline. It is noteworthy that the TOWC group was able to recall previously learned CTA but unable to acquire a new one, the graft-induced recovery of recall is less tissue specific than recovery of acquisition of CTA, and that the IC is highly involved in the evocation aspects of CTA. Supported by DGAPA IN201898.


The comparative neuropsychological approach involves the comparison of brain-damaged human patients with animal models of the same disorders, using tasks designed to assess specific mechanisms in both species. Both rodent and human studies have greatly facilitated when the tasks are as similar as possible for both species, and very difficult when they are not. Many researchers have succeeded in devising such task equivalence, for example, the use of "junk objects" in the Wisconsin General Test Apparatus or, more recently, the presentation of computer graphic stimuli on a VDU/touchscreen apparatus in primates and patient groups. Unfortunately, few tasks have been designed for the rat with this approach in mind. This is perhaps surprising given that the rat is, in psychology at least, by far the most popular and convenient laboratory animal.

Thus, we have recently developed a touchscreen procedure for the rat using computer generated visual stimuli. In addition to inter-species compatibility, other advantages of this technique include the nature of the response, the rat being able to interact directly with the stimulus by nose-pokes to the VDU screen; the ease with which rats can be trained to respond in this manner; and the ability to collect data on a wide range of performance measures. These points will be discussed and data will be presented to illustrate the use of a touch-sensitive screen for rats on various tasks, including object discrimination and serial reversal learning. 8-pair concurrent visual discrimination learning, spatial and non-spatial delayed non-matching to sample and visuospatial conditional rule learning.

494.10 RATS' VISUAL MEMORY IN A COMPUTER-CONTROLLED TESTING ENVIRONMENT. B.A. Gappan*, Department of Psychology, Reading University, Reading RG 2AL, UK and M.J. Eccott, Department of Psychology, Durham University, Durham DH1 3LE, UK.

Rats, of the Hooded Lister and Dark Agouti strains, were trained in a fully automated setting: a Y-maze each of whose arms terminates in a pair of adjacent monochromatic VGA screens, with a food dispenser between them. The abstract stimuli include internally complex, wide-angle displays - "scenes" analogous to those whose processing is disrupted by hippocampal system lesions. The stimuli - = - and, = - are used to create very, localized, homogeneous "objects". Displays can incorporate movement and brightness fluctuation to enhance their salience. We report rats' performance in tests of associative reference memory (concurrent discrimination among pairs of displays) and working memory (matching or non-matching).

494.12 MEMORY FOR FOOD REWARD MAGNITUDE: THE ROLE OF THE AGRANULAR INSULAR CORTEX. W.E. DeCotis, B.P. Kessner* and J.M. Williams, Department of Psychology, University of Utah, Salt Lake City, UT 84112.

Memory for magnitude of reinforcement was assessed in rats using a go, no-go task. During the task's study phase rats were given a piece of cereal comprised of either 25% or 50% sugar. For all trials, one of the cereal types was designated positive, the other negative. On the ensuing test phase the rat was presented with an object which covered a food well. If a positive food reward was given during the study phase, a second food reward was placed beneath the object. No food reward was placed under the object if the study phase consisted of a negative food reward. Latency to object displacement was used as the measure of performance. Following the establishment of a significant difference between latency to approach the object with reward compared to latency to approach the object without reward, rats were given either medial agranular cortex, agranular insular cortex, infralimbic/per- limbic cortex or sham control lesions. Sham controls excepted, all lesion groups showed post-surgery impairment followed by recovery of performance. Furthermore, animals transferred to a new set of cereal comprised of either 25% or 50% sugar. Thus, independent of lesion type, all post-surgery animals were able to perceive differences between magnitudes of reward. Trials consisting of 10 and 20 second delays between the study and test phases were then introduced. Only agranular insular lesioned animals showed significant impairment at each delay. These results demonstrate that lesions to the agranular insular cortex in rats produce accelerated forgetting of reward magnitude and suggest that this structure plays a significant role in memory for affect.
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494.13 NEGATIVE PATTERNING DISCRIMINATION PERFORMANCE IS IMPAIRED IN RATS WITH BILATERAL QUINOLICAL ACID LESIONS OF THE NUCLEUS ACCUMBENS AND MAGNOCELLULARIS. S. Culver, E. McNamara, J. E. Jack, and T. S. Chi. Dept. of Psychology, University of New Mexico, Albuquerque, NM 87131.

It was hypothesized that NBM lesions would selectively impair the ability to learn conditional or configural associations while sparing the ability to learn simple, or elemental, associations (see Sutherland & Rudy, 1989). To test this hypothesis, rats with selective NBM or sham lesions of the NBM and were subsequently tested in a negative patterning operant discrimination task. In this task, rats received reinforcement for depressing an operant lever in the presence of either a light (L) or tone (T) but, are not reinforced (-) in the presence of a compound stimulus comprised of the light and tone presented together (LT). Animals that learn the reinforcement values of the elements L and T are determined by whether these stimuli occur separately (L- versus T-) or simultaneously (LT-). Thus, learning to withhold responses to LT- requires the formation of a configural representation of the compound cue in relation to the representations of its constituent elements. Based on evidence suggesting that simple associative learning remains intact in NBM-lesioned animals, it was hypothesized that these animals would learn to respond normally to the elements L- and T-. It was further hypothesized, however, that NBM-lesioned animals would be unable to form the configural representation of LT- and would continue to respond in the presence of this nonreinforced compound stimulus. Data support these hypotheses. All animals were able to discriminate between the presence and absence of the reinforced elements L- and T-. It is speculated that the ability to solve simple associative discriminations is unimpaired in NBM-lesioned animals. In contrast, negative patterning discrimination performance was impaired in NBM-lesioned animals; lesioned animals made significantly more LT- responses across training sessions than controls (p<.05), although behavioral recovery eventually ensued. Results suggest a selective involvement of the NBM in the acquisition of configural but not simple associative tasks. (Supported by NIH R01 NS-27695.)

494.15 EFFECTS OF MEDIAL SEPTAL LESIONS ON DELAYED-GO/NO-GO RESPONSE ALTERNATION IN RATS. R. Rumen. Psychology Department, Santa Clara University, Santa Clara, CA 95053.

In an earlier study (Nanam and Quaranta, 1990) we found that medial septal lesions impaired a two-stage delay operant alternation task in rats. We concluded that the impairment was due to a disruption of working memory. However, a "spatial" interpretation of the data could not be ruled out. Subsequently (Nanam and Kim, 1992), we found that medial septal lesions facilitated performance on a cue go/no-go discrimination task with a delay between the cue and the required response. This was not the case with the previously used go/no-go working memory paradigm. Hence, the lesion could not have produced a general working-memory impairment. This finding together, suggested to us that the medial septal lesions impaired "spatial" memory, and that the two stages of the task led to a compensatory enhancement of "stimulus" working memory. If this were true, then the lesions should impair a go/no-go task based on response working memory. The current experiment tested this hypothesis. Rats (12 with medial septal lesions and 12 with sham operations) were tested on a discrete-trial operant go/no-go response alternation task without cues. The rats were first tested for 20 days without a delay contingency, followed by 30 days of testing with a 15-s delay between the cue and the required response. The shuttle lesions did not impair performance at the 0-s delay (p>0.05). Both groups achieved 85% correct and acquired the basic requirements of the task. However, when the delay contingency was added, the medial septal lesions impaired performance (p<0.025). By the end of testing, the controls averaged 75% correct while the lesioned rats averaged 67% correct.

As the go/no-go task does not require spatial processing, the best explanation for all of these findings is an impairment on L-R delayed alternation and delayed go/no-go response alternation, but facilitation on a delayed but cue go/no-go discrimination is that the medial septal lesions produce an impairment in "response" working memory. Such an impairment may result in a compensatory enhancement in the processing of salient environmental cues to guide behavior and improve performance on cue-task.

494.17 LATERAL DORSAL NUCLEUS OF THE THALAMUS CONTRIBUTES TO SPATIAL LEARNING. M. D. Palmer and R. J. Sutherland. Dept. of Psychology, University of New Mexico, Albuquerque, NM 87131.

The lateral dorsal nucleus (LD) of the thalamus has reciprocal connections with retrohippocampal areas. Recent electrophysiological work implicates LD as playing an important role in the spatial navigation process, especially in encoding head direction (Mizumori, Kuhar & Amaral, 1992). The present experiment was designed to investigate LD's role in spatial learning.

Eight rats received electrolytic lesions of LD (coordinates: -2.6, +2.6, -2.6, 5.7) of 15mA for 1s. Sixteen rats served as sham controls, which were naive to the water task. We used a moving platform version of the water task in which the submerged platform was moved to a new position on each subsequent day, but remains in the same position over the course of trials. The LD rats displayed a significant deficit in learning relative to the sham controls.

We also report on acquisition of a negative patterning discrimination and a light-tone discrimination in an operant task.


The nucleus basalis magnocellularis (NBM) is the major cholinergic projection to frontal cortex and its depletion appears to modulate cortical neuronal activity. Lesions of the NBM produce deficits in radial arm maze performance and morphological alterations in frontoparietal cortex. We have assessed selective attention and noradrenergic receptor binding in frontal cortex of rats with bilateral lesions of the NBM. Nineteen rats received either bilateral NBM or sham lesions of the NBM. Two months after surgery, rats were trained in a block task. While sham-lesioned rats showed blocking, suppressing significantly more to a meaningful cue than to a non-meaningful cue. NBM-lesioned rats did not show blocking, suppressing less to both cues. The groups performed comparably on a passive avoidance task, suggesting that deficits on the block task were due to attentional changes rather than a learning deficit. Three months after surgery, rats were killed and their brains removed. Sections were incubated with either [3H]quinoxalin-3-(4H)-side or [3H]desmethyrimipramine (DMI) to assess noradrenergic function and processed for autoradiography. Quantitative receptor binding revealed a 28% decrease in DMI binding and a 24% decrease in DMI binding in frontal cortex of lesioned rats. Thus, the NBM appears to modulate noradrenergic function in frontal cortex, and to alter the behavior and cortical morphology through the noradrenergic system.

494.16 RETROGRADE AND ANTEROGRADE SPATIAL MEMORY IMPAIRMENTS FOLLOWING RADIO-FREQUENCY-LEVEL QUESIONS TO THE LATERAL INTERNAL MEDULLARY LAMINA. L.M. Savage, A. S. A. Bassett, and P. J. Langlais. VA Medical Center San Diego, CA 92169.

Rats were pre-trained on a Non-Matching-To-Position (NMT) task in the T-maze. After initial acquisition (90% correct for 2 sessions) subjects were randomly assigned to two treatment conditions: (A) radio-frequency lesions to the lateral Internal Medullary Lamina (IML), or (B) probe placement in the same location (sham). Following recovery from surgery, the IML-lesioned rats took significantly more trials than sham rats to re-acquire the criterion. This suggests that lesions to the lateral IML region produce retrograde deficits. Once subjects re-mastered the NMT task they were re-introduced to the water and effects of lesions were tested. Although the performance of IML-lesioned rats at the shortest delay (4 s) was equivalent to shams, they required longer delays (30, 60, 90 and 90 s) to complete the task. Anterograde learning and memory was subsequently assessed using the Morris water maze. Again, IML-lesioned rats demonstrated impaired spatial abilities—their latency to find the location of the hidden platform was significantly longer than shams. However, they were able to decrease their latency to find the location of the visual platform. IML-lesioned rats, relative to shams, demonstrated no deficits in learning a single trial passive avoidance task. These results suggest that lesions to the IML region produce a range of spatial deficits that parallel the ptyalin-induced thiaamine deficiency model of Wernicke-Korsakoff syndrome. Funded by a VA Merit Award to PJL.

494.18 IBOTENATE LESIONS OF RAT NUCLEUS REUNIBENS THALAMI FAIL TO IMPAIR SPATIAL LEARNING AND MEMORY. M.Dollman-van der Weel*, R.O.M. Monte, and M.O. Winter. Graduate School of Neurosciences, Dept. Anatomy and Embryology, Vrije University, Amsterdam, The Netherlands, "Centre for Neuroscience, University of Edinburgh, Scotland.

Monosynaptic projections from the thalamus to the cerebral cortex may enable it to modulate activity in the entorhinal-hippocampal circuit and, hence, play a role in learning and memory. We explored this idea using the watermaze (fixed hidden platform, 18 spatial training trials, transfer test, 4 cue trials). The reuniens (RE, n=12) was lesioned with ibotenate acid and the behavioral effects were compared to Unoperated (Uop, n=6) and sham-lesioned controls (SH, n=11), medial dorsal nucleus (MD, n6) and hippocampal-dentate gyrus lesioned rats (HDG, n=6). ANOVAs revealed overall Groups effects in both training and the transfer test (p<.001), but, despite a trend towards poor performance by the RE group, pairwise comparisons (Dunnnett's test) revealed that only the MD and HDG groups were impaired relative to the SH group during training (p<.01 and p<.025 respectively) and only the IDG group was impaired during the transfer test (p<.025). The cue test revealed a trend towards poorer performance in the MD group (p>0.05). Our protocol was, therefore, sufficiently sensitive to detect hippocampal dysfunction in a spatial navigation and memory, but showed only a non-significant trend towards impairment following damage to the reuniens.

In summary, the presumed modulatory input from the reuniens to the entorhinal-hippocampal circuit was only a subtle effect on spatial learning. Its function may be better revealed using other learning and memory paradigms.
494.19

Rats with bilateral electrolytic or sham lesions of the mediodorsal thalamic nucleus (MD) were tested on a battery of six object-memory tasks, which resemble those which have been used in the study of amnesia in humans and monkeys: (1) simple two-choice object discrimination, (2) discrimination reversal, (3) 8-pair concurrent object discrimination, (4) two-choice switch-to-sample (DNMS) with retention delays of 4, 5, 30, 60, 120 s, (5) DNMS with lists of 3, 5, and 7 samples, and (6) order discrimination. Each task made use of the same apparatus as the monkey versions of these tasks, each one used objects as test stimuli. All testing was conducted posturgery.

Relative to control rats, the rats with mediodorsal thalamic lesions were impaired at learning the simple object discrimination, but they did significantly more trials to master both the object reversal and the concurrent object discrimination tasks. The DNMS testing revealed that the rats with MD lesions were impaired at all delays of 15s or longer, and there was an overall group impairment across the three list lengths. There was no significant difference between the controls and MD-lesioned rats on the temporal-order discrimination task. The results from this battery of memory tasks will help in fully characterizing the nature of the memory deficits associated with medial diencephalic lesions in the rat and allow for direct comparisons to similar experiments in monkeys.

494.20
MEMORY IMPAIRMENT FOLLOWING LESIONS TO THE ANTERIOR NUCLEI OF THE THALAMUS. V. Szikla*, F. Ler, and M. Petrides. McGill University, Montreal, Quebec, Canada.

In the present study, rats with lesions to the anterior thalamic nuclei (ATN), the fornix (FX) or a control operation (OC) were trained on an eight-arm radial maze under two conditions. In the first condition, there was a 20 sec delay between choices, in the second, there was no intradelay. Animals with lesions to the FX improved significantly when there were no delays between choices but the performance of this group was significantly impaired in comparison with the OC animals under both conditions. The overall performance of the ATN group was significantly worse than that of the OC group and, in addition, did not improve significantly with the removal of the intradelay. An analysis of the distribution of errors made in the delay condition suggests that the nature of the memory deficit following lesions to the ATN and the FX might be different. Taken together, these findings suggest that restricted lesions to the ATN are sufficient to produce a working memory deficit on the radial maze under the conditions used in this study.

494.21

The tuberomamillary nucleus (TM) is located in the posterior part of the hypothalamus and provides the only source of neural histamine in the CNS. Our experiments performed with unilateral lesions of the TM region provide evidence for an involvement of the TM system in reinforcement mechanisms. Unilateral destruction of the TM with DC or ibotenic acid was found to increase the rate of lateral hypothalamic self-stimulation ipsilateral to the lesion site, suggesting that the TM may function as a reinforcement inhibiting neural substrate. Experiments performed with bilateral lesions of the TM region provide evidence for a role of the TM in learning and memory processes. Adult (3-month-old) and aged (31-month-old) rats with bilateral DC lesions in the TM region were tested along with sham-lesioned controls on the one-trial step-through inhibitory avoidance task and on a spatial discrimination test in a water-filled T-maze. Bilateral lesions of the TM led to significantly longer retention latencies in the step-through task and improved long-term retention in the water maze test in both adult and aged rats, indicative of superior learning. Thus, lesions of the TM may have a facilitatory e ffect on learning and mnemonic functioning, which is possibly related to a lesion-induced disinhibition or facilitation of rein-forcement ("stamping-in") processes. Supported by DFG.

494.22
INVESTIGATION OF NEURAL AREAS CONTROLLING CONDITIONED TASTE AVersions IN RATS. EFFECTS OF COOLING THE AREA POSTREMIA. Y. Wang, D. G. Lavond, & R. C. Chambers*. Department of Psychology USC, Los Angeles, CA 90089.

Four studies were designed to determine the feasibility of using cooling to investigate neural mechanisms of conditioned taste aversions (CTA). Study 1 was designed to determine whether cooling the arms postremia (AP) can induce a CTA. After 13 rats were given access to a sucrose solution, the AP of 7 of the rats was cooled for 1 hour and the AP of 6 was not cooled. Starting 2 days later, all rats were given daily extinction trials until their acquisition day consumption levels were restored. Cooling the AP induced a CTA; the rats whose AP were cooled showed a decrease in sucrose consumption but the non-cooled group did not. Study 2 was designed to determine whether precooling to cooling of the AP could attenuate the CTA induced by cooling the AP. In 7 rats, the AP was cooled daily for 7 days and in another 7 rats the AP was not cooled. On acquisition day, the AP of all rats was cooled after consumption of sucrose solution. Precooling to cooling attenuated the aversion induced by cooling; the non-precooled group showed a decrease in sucrose consumption but the precooling group did not. Study 3 was designed to determine whether cooling the AP could be used to block a CTA induced by LCi. All rats were preiced to cooling. After sucrose consumption, the AP of 6 rats was cooled just prior to injection of LCi and was kept cooled for 1 hour. Another 7 rats received LCi only. Cooling the AP attenuated the aversion induced by LCi rats exposed to cooling during LCi stimulation acquired a weaker aversion than those not exposed. Study 4 was designed to determine whether cooling the AP could block a CTA induced by sponspheine (Apo). After sucrose consumption the AP of 8 rats was cooled just prior to injection of Apo and was kept cooled for 1 hour. Another 7 rats received Apo only. Cooling had no effect on the aversion induced by Apo. The results of these studies, attenuated aversion induced by LCi but not sponspheine, replicate those of studies that have used permanent lesion techniques. Supported by USC FRP.

494.23
DIFFERENT SUBCORICAL AREAS SUBERVE SPATIAL BUT NOT VISUAL DISCRIMINATION PERFORMANCE IN INBRED MICE. M. Ammendt, P. Reile and P. Rosig-Arnau**. Inst. of Psychobiology and Psychopharmacology and * Department of Psychology, University of Rome "La Sapienza", Rome, Italy.

C57BL/6 (C57) and DBA/2 (DBA) inbred mice differently perform spatial tasks with C57 doing better than DBA at such learning. Hippocampal lesions have been shown to impair performance in both strains while amygdaloid and frontal cortex lesions have a deleterious effect only in the "high learner" strain C57. The aim of the present study was to examine whether the genotype-dependent involvement of limbic structures remains the same across tasks which make either the spatial or the visual modality relevant for discriminating the set of bailed arms. C57 and DBA mice were tested in the standard (experiment 1) or in the visually-cued (experiment 2) version of the four-baited path task in a radial eight-arm maze.

Results show that, when the spatial modality was relevant for identifying the correct arms, both hippocampal and amygdaloid lesions impaired performance in C57 while hippocampal but not amygdaloid lesions impaired performance in DBA. Conversely, when the visual modality was relevant, the two strains performed the task in the same way, and their performance was, but they took hippocampal lesions only. Spatial but not visual discriminative learning is therefore selectively based upon the involvement of different subcortical areas in the two strains considered.

494.24
ASYMMETRICAL EFFECTS OF LESIONS IN FR2 ON LEFT-RIGHT RESPONSE DIFFERENTIATION IN THE RAT. M. Newcorn*, D. Chmiel, Jc, and S. Axelrod. College, Buffalo, NY 14208; 2 SUNY at Buffalo, Buffalo, NY 14214.

We compared rats with left or right lesions in supplementary motor area Fr2 (Zilles), and sham operates, on a left-right response differentiation (LRD) water-maze task given after pair experience with a control task in the same apparatus. We found that (1) rats exhibited an overall left-turning bias during acquisition, (2) the bias was particularly pronounced on the LRD task, and (3) right-lesioned rats performed better than left-lesioned rats.

Overall, our results provide evidence of a consistent cerebral asymmetry in the rat. The left-turning bias replicates earlier findings in our laboratory and points to asymmetrical motor activation favoring the right hemisphere during these water escape tasks. Nonetheless, a lesion in the right Fr2 left our rats better able to acquire an LRD task than a lesion in the left Fr2. We have previously shown that extensive right hemisphere/occipital, compared to left decortication, improves performance on the LRD task when it was similarly presented as a second learning experience. The present finding adds to localize the relevant asymmetry to area Fr2, and suggests that it is motor/attentional in nature.

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495.1 DIFFERENTIAL INFLUENCE OF HIPPOCAMPAL SUBFIELDS CA1 AND CA3 IN ODOR DISCRIMINATION. U.S. Hest, G. Lynch, and C.M. Gall. Dept. of Psychology, Univ. of CA, Irvine 92851.

Expression of the c-fos gene is a useful marker of neuronal activity. In the present study, levels of c-fos mRNA were evaluated in order to determine discrete areas that underlie odor discrimination. Rats were trained on one odor pair in a familiar chamber with six odor alleys. Oders were emitted from any two random odor ports on a given trial and rats had not c-fos mRNA at the nose site and to avoid the negative response (response to which resulted in a strobe flash). Control rats (i.e. nosepoke controls) performed praxial tasks in the same apparatus with no odor presentation. c-fos mRNA was quantified using in situ hybridization of 35-S-RNA probes. Hybridization to c-fos mRNA was not detectable in rats performing the task (learners) as compared to control

495.2 GLUCOSE FACILITATES ACQUISITION AND REDUCES HIPPOCAMPAL DEFICIT IN THE MORRIS WATER MAZE IN RATS. R.W. Skelton & S.R. Russ. Psychology, U. Victoria, Box 3065, Victoria, B.C. V8W 3P6 CANADA

Post-trial glucose has well-known facilitatory effects on retention of inhibitory conditioning. Increases in blood glucose levels have been proposed to underlying the mnemonic facilitation produced by epi- nphrine, norepinephrine and epinephrine. The present study examined the effects of pre-trial glucose on performance of rats recovering from bilateral transections of the perforant path (a major cortico-hippocampal-cortical pathway).

During initial acquisition, glucose (100 mg/kg) was administered for 10 days 9 min before or after 2-trial daily sessions with a submerged platform in a novel location. Probe trials were given as the second trials of Days 5 and 10, and an undrugged probe trial was given after a 5 day undrugged retention interval. After such pretreatment, rats were given an intertrial delay to the test platform and allowed 5 days to recover. They were then tested for 7 days with the platform in the original location and 7 days with the platform in the opposite quadrant. Glucose (100 mg/kg) was injected 15 min prior to each day was mg of 4-mercaptoethanol.


Previous studies with rodents showing alterations in both spatial learning and LTP following injections of somatostatin (SS-14) or other somatostatin analogs suggest that specific subfields of the hippocampus (CA1, CA3) are involved in the acquisition. In the present study, the hypothesis that hippocampal SS-14 could play a role in spatial mapping processes. However we reported that, although intrahippocampal SS-14 injections facilitated the acquisition (regular trials) of spatial discriminations (two overlapping pairs (A+B) vs. (C+D)) was also impaired performance during probe trials (B+C) aimed at having relational representations. Guillois et al., Psychology 21:4, 1993. In the present study the aim was to determine whether this latter impairment was due to a deficit of relational encoding during the acquisition of the task or, alternatively, to a retrieval deficit (fragment fitting) occurring only when testing was shifted on probe trials. Accordingly, SS-14 or artificial CSF was injected either before regular probe trials, or both. As in our previous study, injections of SS-14 before both regular and probe trials facilitated regular trials significantly and facilitated probe trials. Injections of SS-14 before only regular trials produced the same pattern of results. In contrast, injections of SS-14 before only probe trials did not yield any behavioral effect. Thus, injections of SS-14 into the hippocampus appear to disrupt relational representations that, in normal mice, would occur during the acquisition of the task. This SS-14-induced deficit of relational representations could account for the facilitation of acquisition (by use of individual representations of each pair). However, other results along with the observation that c-fos expression produces a large impairment on regular trials seem difficult to explain by the relational theory alone. Taken together, these results suggest that both spatial mapping and the formation of relational representations are two separate HPC-dependent functions.

495.4 DENTATE GAMMA DEATH AND SPATIAL LEARNING IMPAIRMENT AFTER CORTICOSTEROID REMOVAL IN YOUNG AND MIDDLE-AGED RATS. C.D. Conrad, D.L. Boy, Neurosci., Univ. Ill., Champaign, IL 61820.

We investigated the functional and behavioral implications of chronic corticosteroid removal in young and middle-aged rats. Pre-pubertal rats were placed in these groups: adrenalectomized (ADX) with no hormone replacement; ADX given corticosterone chronically, chCORT; ADX given corticosterone acutely at time of Morris water maze at 12 weeks old, aCORT. 13 month old rats were ADX or SHAM operated only. All rats were run on the Morris water maze 12 weeks after surgery for 11 days after which, they were sacrificed and brains saved for histological analysis.

The results showed that prolonged corticosterone absence caused major damage to the dentate gyrus and impairment on the Morris water maze. The chCORT rats had little dentate gyrus cell loss and were as efficient as the SHAMs in the Morris water maze performance whereas the aCORT rats had dentate gyrus cell loss and were impaired in the spatial acquisition task. Middle-aged ADX rats lost cells only in the dorsal blade of the dentate gyrus but they did not show a greater learning impairment in the Morris water maze relative to the middle-aged SHAMs. These results indicate that corticosteroid absence is trophic for the dentate gyrus and that substantial dentate gyrus damage impairs spatial learning.

495.5 BEHAVIORAL AND ANATOMICAL EFFECTS OF 192-SAPORIN AND ANTI-DH-SAPORIN PASSIVE AVOIDANCE, CONDITIONED FREEZING AND OPEN FIELD ACTIVITY. R.G. West, T.G. Berber, D.A. Lepel, M.J. Pickel & D. Roberts. VASC & Vanderbilt University, Nashville, TN 37212 & The Whitter Institute, La Jolla, CA 92037.

Analysis of the behavioral function of the cholinergic basal forebrain (CBF) has been benefited from the isolation of the immunotoxin, 192-saporin, that selectively destroys neurons expressing the low affinity NGF receptor (p75). Animals with high CBF lesions are profoundly impaired in a variety of behaviors including passive avoidance. Since the somatodendritic projections innervate the hippocampus, thalamus, and basal forebrain, the CBF and many of its targets (hippocampus, neocortex), we sought to compare rats injected intravenicularly with 192-sap (N=8) to controls (N=8) and rats treated with another immunotoxin, anti-DH-saporin (N=8), to determine the effects that CBF lesioning (cholinergic & noradrenergic) is expressing the enzyme, dopamine B-hydroxylase. Rats injected with anti-DH-saporin were slower to regain weight after surgery than rats injected with 192-saporin (p=0.002). Anti-DH-saporin rats were significantly inhibited in a conditioned fear paradigm relative to 192-sap treated rats (p=0.003). In a step-through passive avoidance task, anti-DH-saporin treated rats behaved similar to controls showing none of the impairment seen in the 192-sap treated rats (p=0.001). In the open field, there was a nonsignificant trend for increased activity among 192-sap treated rats compared to the controls and anti-DH-saporin treated rats. Immunocytochemical staining for choline acetyltransferase (ChAT) showed near complete (90%) loss of ChAT-positive neurons of the CBF in 192-saporin treated rats compared to controls. Anti-DH-saporin treated rats showed about 50% decrease in p75/ChAT neurons in the CBF and near complete (90%) loss of tyrosine hyroxydase (TH)-positive neurons from the noradrenergic and adrenergic brainstem nuclei. Dopaminergic TH neurons were unaffected in the substantia nigra and ventral tegmental area. These results confirm that 192-sap and anti-DH-saporin produce the expected selective lesions that animals prepared with these agents are useful in analysis of the behavioral function of CNS cholinergic and noradrenergic neural systems.


The available evidence supports the hypothesis that the immunotoxin 192 IgG-saporin selectively destroys the cholinergic neurons bearing p75 NGR receptors. Heckers et al. (1984) have shown that lesions in the basal forebrain destroyed the cholinergic projections to the cortex and spared the cholinergic neurons projecting to the amygdala and thalamic nuclei (which do not bear NGR receptors). As cortical cholinergic afferents have been assumed to mediate the subjects’ ability to detect and process behavioral significant stimuli, the effects of 192 IgG-saporin-induced lesions of the cholinergic basal forebrain on behavioral vigilance were examined. Rats received infusions of 192 IgG-saporin (0.20 µg/0.5 µhemisphere) after they were trained to criterion in an operant vigilance task. This task requires the animals to discriminate between signal (central panel light presented for 25, 50 or 500 sec duration) and nonsense stimuli. When a correct signal rejection was rewarded, while misses and false alarms were not. Following the post-learning retraining period, animals were treated with a benzoazepine receptor agonist (cloroxazepoxide, 1, 5, 3 mg/kg, i.p.), and partial inverse agonist (vaclopride, 5, 1, 2 µg/kg, i.p.) to test the hypothesis that, in lesioned animals, the detrimental effects of the agonist are more potent than in control animals, and that the partial inverse agonist attenuates the lesion-induced impairments in vigilance performance.
495.7
19 Ig-saporin lesions of basal forebrain cholinergic cells effects on learning and memory in rats. M. C. Baxter1, B. J. Back2, A. A. Chiba3, L. Chai, R. C. Wilson, and M. Gallagher1,4
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The development of 19 Ig-saporin, which acts as a specific cholinergic neuronal when infused directly into basal forebrain nuclei, permits direct assessment of the role of basal forebrain cholinergic cells in cognitive function. Male Long Evans rats, 2-3 months old, received infusions of 19 Ig-saporin (IES) or vehicle (CON) into either the medial septal area (MSA) or substantia innominata (SI). Place discrimination in the Morris water maze assessed spatial reference memory, and a 2-trial matching-to-place task in the Morris water maze assessed spatial working memory. MSA-CON and SI-CON rats did not differ in behavioral performance and were pooled for statistical analysis. MSA-LES and SI-LES rats were not impaired relative to CON rats in acquisition of the place discrimination. MSA-LES and SI-LES rats were impaired relative to CON rats in performance of the working memory task; however there was no obvious delay-dependence of the deficit at the delays tested (delays ranged from 30 seconds to 3 hours), suggesting that this deficit may not be purely neomeric in nature. This pattern of results calls into question the central role postulated for cholinergic neurons of the MSA in learning and memory. (Supported by NIA Grant PO1-AD09973 to MG and an NSF Predoctoral Fellowship to MGR.)

495.8
Substantia innominata lesions impair incremental attentional processing in a Pavlovian conditioning paradigm. A. A. Chiba1, L. S. Han1, E. C. Holland2, and M. Gallagher1,4
1Department of Psychology, Univ. of North Carolina, Chapel Hill, NC 27599; 2Department of Psychology, Duke University, Durham, NC 27706.

Holland and Gallagher (1990) demonstrated that a central nucleus of the amygdala (CN) in incrementing attention based on associations formed during appetitive conditioning. Furthermore, recent evidence indicates that habituation processes by CN may be mediated through the corticopetal system located in the substantia innominata (SI), a component of the basal forebrain system in the rat brain.

An associative learning paradigm was used to examine the role of the SI in modulating attention. Each rat was exposed to conditioned stimuli (CS) that were either consistent or inconsistent predictors of subsequent CAs. Intact control rats maintained higher levels of attention to a CS when it was an inconsistent predictor of subsequent CAs, whereas SI-lesioned rats failed to show this enhanced attention. Thus, SI-lesioned rats displayed an impairment in incrementing attention equivalent to that previously demonstrated by CN-lesioned rats. These data support the notion that CN attentional modulation is dependent on the projections of the CN to the SI corticopetal cholinergic system. Based on this hypothesis, further experimentation using selective cholinergic lesions of the corticopetal system is being pursued within the behavioral paradigm. (Supported by an award from the Human Frontier Science Research Program.)

495.9
c-fos expression following paired or unpaired cutaneous nerve stimuli: specificity related to LTP of spinal reflexes. R. O. Durieux*, Dept. of Physiology, SUNY Health Science Center, Syracuse, NY 13210.

In order to help define the neural circuits involved in two forms of associative reflex potentiation, spinal neurons expressing c-fos were examined following various qualities of cutaneous nerve stimuli. Previous work from this laboratory has shown that in the spinal caudal preparation when two cutaneous nerves are stimulated in specific "paired" sequences, flexion reflexes evoked by stimulating one of the nerves exhibited LTP (Long-Term Potentiation) that lasts for hours. These reflex changes obey the general rules established for learning and memory behavior in intact animals (Nature, 319:211, 1986). Thus, these spinal reflex changes represent a specific model for investigating neural mechanisms of elementary forms of learning and memory behavior. The experiments reported here compare the number and locations of fos labeled neurons in the spinal cords of control and experimental animals (one or two nerve stimuli), animals given 30 "unpaired" cutaneous nerve stimuli, and animals given 30 "paired" cutaneous nerve stimuli presentations. fos labeling was differentially expressed among these groups with few neurons labeled in control animals and the greatest number of labeled neurons in "paired" animals. Furthermore, two methods of "pairing" resulted in concentrations of labeled neurons in different spinal cord regions. The paradigm that preferentially potentiated spinal circuits activated by large myelinated superficial cutaneous nerve fibers labeled neurons primarily in deep laminar (VII) of spinal cord. The paradigm that preferentially potentiated spinal circuits activated by small myelinated superficial cutaneous nerve fibers labeled neurons primarily in more dorsal laminar (IV-V). The results are consistent with the hypothesis that learning and memory result from alterations in the synaptic strengths of specific sets of synapses. Supported by NSF grant IBN 922020K.

495.10
Long term potentiation (LTP) of pyramidal neurons in layer five of the cat motor cortex. Akhita Kimura*, Francesco Mela, Marcello A. Cara and Hiroshi Asanuma. The Rockefeller University, New York, N.Y. 10021.

Long term potentiation is supposed to play a crucial role in learning new skilled movements (Asanuma and Keller, 1991). A series of experiments revealed ubiquitous existence of LTP in various structures involved in execution of skilled movements. In this study, generation of LTP within the motor cortex was studied using intracellular recording and labeling (biocytin) techniques under Nembutal anesthesia. Intracortical microstimulation (ICMS) was delivered in the superficial layers and recording were obtained in layer V of the motor cortex. Of 20 labeled cells in layer V, 6 cells showed short lasting potentiation (1-4 min, 160-240% of control EPSP) immediately after tetanic ICMS (100-200 Hz, 10-30 sec) which was followed by steady potentiation (130-150% of the control) lasting until the electrode went out of the cell (7-28 min). In 12 labeled cells, only PTP (120-270% of the control) was observed. The duration of the PTP was from one to 4 minutes. All the labeled cells were pyramidal neurons. The effective stimulating sites inducing LTP were located immediately superficial to the labeled neurons. These results suggest that plasticity exists within the intracortical connections of the motor cortex that may be involved in learning of new motor skills.

495.11
Inhibition of nitric oxide reduces hippocampal mediation of place learning in the rat. G. Wörlein, B. Gustafson, P. Ernsatz and J. Mogensen*. Laboratory of Neuropsychiatry, University Hospital 4102, Copenhagen, Denmark.

In Experiment 1 the behavioural consequences of transection of the fimbria-fornix were investigated in animals that had acquired a place learning task after a control pretreatment or a pretreatment period during which near-total inhibition of NOS (the nitric oxide synthesizing enzyme) had been accomplished. While the lesion significantly impaired task performance in normals, the rats which had acquired the task during NOS inhibition did not reveal a reproducible impairment. In Experiment 2 four groups of rats were studied: two groups received transection of the fimbria-fornix while the other two were subjected to sham surgery. Subsequently, one of the lesioned and one of the sham groups received a period of L-nitro-arginine (L-N-ARG) injections. During the final 5 days of surgery all groups were subjected to training on place learning. While NOS inhibition impaired task acquisition in the sham operated animals, L-N-ARG administration in fimbria-fornix transected rats failed to impair task acquisition. Conclusions: (1) Normal place learning acquisition involves hippocampus associated mechanisms. (2) Under conditions that the task can be acquired by a system that differs from the one mediating task acquisition in normals by receiving reduced contributions from the hippocampus.

495.12
Stress effects on memory and AMPA receptors are abolished by Adrenalectomy. D.M. Diamond*, B.J. Branch, G.M. Rose and G. Tocco. Dept. of Pharmacology, Univ Colo Health Sci Ctr, & VA Med Ctr, Denver, CO and Neurosciences Program, USC, Los Angeles, CA

We have reported that exposure of a rat to an unfamiliar environment (psychological stress) blocked LTP (Psychology, 18:273, 1990, Beh. Brn. Res., in press) and selectively impaired hippocampal-dependent memory (Neurosci. Abst., 19:366, 1993). The present work is a test of the hypothesis that the effects of stress on hippocampal function will be eliminated by adrenalectomy (ADX).

Male rats were transfected on 14-arm radial maze without novel hippocampal-dependent (working memory) and hippocampal-independent (reference memory) components (see Neurosci. Abst., 19:366, 1993 for details). Stress impaired working, but not reference, memory in intact rats. In contrast, stress did not affect on memory in ADX rats. The ADX rats did exhibit other behavioral manifestations of stress (e.g., fecal bolus production). Therefore, the effects of stress on emotionality and cognition (memory) can be dissociated. We have also found that stress produces a hippocampus-specific reduction in ADMA receptor binding in intact, but not in ADX, rats. These findings indicate that stress produces adrenal-dependent effects on hippocampal plasticity, memory and glutamate receptor binding dynamics. Supported by NS, ONR, the VAMC and the McKnight Foundation
LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS XIV


It is well known that benzodiazepines (BZD) produce anterograde amnesia. Recent evidence suggests the amygdaloid complex (AC) GABAergic system mediates BZD-induced amnesia. Lesions of the AC block the impairing effects of a BZD on retention of inhibitory avoidance (IA) behavior. Addition of BIC, a non-competitive antagonist by pre-intracortical (i-AC) infusion of the BZD midazolam (MDZ) and enhanced by intra-AC injection of a BZD antagonist. We recently reported that pre-infusion of the GABAergic antagonist bicuculline monosodium (BIC) into the AC blocks the learning impairing effects of systemically administered midazolam in IA behavior. In general, BZDs impair memory, but not after avertively motivated learning takes place, whereas GABAergic drugs affect retention when administered post-training. Consequently, it is not clear whether these drugs mediate pre-or post-training memory processes. Because BZD-induced behavioral effects are mediated by the GABAA receptor complex, it is likely that BZD-induced memory deficits are also due to antagonizing influences on memory processes. The present experiment examined this implication. Before training in a multiple trial IA task, male Sprague-Dawley rats (250-300g) were injected (ip) with either midazolam (MDZ, 2.0 mg/kg) or vehicle (1.0 μl) was infused bilaterally into the AC. On a 48 hr retention test the performance of the MDZ treated animals was significantly poorer than that of controls. The retention of MDZ-treated animals given intra-AC injections of the lowest dose of BZD (2.0 μmol) was comparable to that of controls, whereas higher doses showed the behavioral impairment. The present results are consistent with other findings indicating that the amygdaloid complex mediates the amnestic effects of BZD on anterograde learning. Furthermore, these data suggest that BZDs impair memory by disrupting post-training processes underlying memory consolidation. Supported by FHS M112526, NIMH & NIDA (ULM).

GABAERGIC-CHOLINERGIC INTERACTION IN THE SEPTAL REGION MEDIATES BOTH ANXIETY AND SPATIAL WORKING MEMORY IN MICE. M.P. Gale*, L. Menzies, L. M. Bellot*, L. Magyar, J. Comportementales Cognitives, CNRS URA 139, Université de Bordeaux 1, 33405 Talence France.

Convergent data suggest that the septal region may constitute an interface between anxiety and working memory (SWM). Using a novel 4-arm radial maze, we have examined the role of cholinergic and gabaergic septal neurons interaction in these two processes. The anxiety level was evaluated by comparison between exploratory activities measured in an elevated plus-maze and in a four hole-board. The SWM was assessed using a sequential alternation procedure achieved in a T-maze. Results indicated that intraseptal infusion of scopolamine (2.5μg/1.2μl) combined with (i.p. injection of diazepam (0.5 mg/kg) in same animals produced an "anxiety-like" effect. Furthermore, SWM was differentially affected by this treatment. Indeed, performance was unaffected when a 30 sec interval was used whereas for a 5 sec intertrial interval alternation scores were reduced below the chance level. In addition, the application of either scopolamine or diazepam alone did not induce any changes in locomotion. Therefore, our data suggest that a cholinergic/gabaergic septal mechanism mediates both the anxiety phenomenon and SWM. This interpretation is consistent with hypothesis that the cholinergic septo-hippocampal pathway promotes anxiety (GRAY, J., Oxford University Press, Oxford, 1982.) Thus, decrease in SWM abilities observed after modulating activity of these structures seems to be the consequence of release from an anxious response, which will increase vulnerability to proactive interference rather than an accelerating forgetting.

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Previously reported by us (Hippocampus 1(10) mg/kg sc of MK-801 produced chronic memory impairment in adult male mice (Brown-Watters et al., 1999). Here, we confirm in Harlan ICAN rats that MK-801(10 mg/kg sc) chronically impairs acquisition performance on a spatial learning task, and, in addition, produces neuronal necrosis confined to the posterior hippocampus. In Exp. I, rats were randomly assigned to one of two groups: saline or saline + MK-801 (10 mg/kg sc) in a one-trial training session. The criterion was to reach a distance of 10 cm from the platform within 120 s. The results indicate that rats in the saline group were able to learn the task, as reflected by their ability to find the platform in the subsequent sessions. In contrast, rats in the MK-801 group showed no improvement in their ability to locate the platform over the course of the training sessions. These findings suggest that MK-801 may have a profound effect on the hippocampus, potentially impairing learning and memory processes in these animals.

495.20 EVIDENCE SHOWING THAT HIPPOCAMPAL PROTEIN KINASE C ACTIVITY APPEARS TO BE MORE LINKED TO LEARNING ABILITY THAN TO LEVEL OF TRAINING. J. Nogues, J. Micheur* and R. Jaufret Lab. Neurosciences Comportementales et Cognitives, URA CNRS 339, Univ. Bordeaux I, Av. des facultés, 33405 Talence Cedex, France.

We have previously shown a decrease in hippocampal cytosolic PKC activity following partial learning of a reference memory task in a radial maze (Nogues et al., 1999). In the present study, we examined whether the expression of PKC activity remained unchanged, but was negatively correlated with performance level. Since this result did not fit with the translocation hypothesis, this study was designed to examine the effects of an increased level of training on PKC activity in the hippocampus. Mice were trained in a spatial temporal discrimination task performed in an 8- arm radial maze for 2, 5, or 12 sessions. Then, PKC activity was measured in membrane and cytosolic compartments of the hippocampus. Behavioral results showed a slower rate of acquisition as compared to our previous studies. Biochemical results did show any significant decrease in cytosolic PKC activity for any group. Nevertheless, membrane bound PKC activity correlated significantly with the number of reference memory errors at the third day of training (r=0.74; p<0.001). Firstly, these data show that the decrease in cytosolic PKC activity is not cumulative across sessions. Secondly, they confirm the correlation between membrane-bound activity and performance accuracy. Further statistical analysis on all animals tested in our previous experiments (n=77), shows that the amplitude of the PKC cytosolic decrease is correlated with performance accuracy on the second day of training (r=0.24; p=0.02; one-tail).

Tackled together, these observations support the idea that the membrane-bound PKC activity derives from a pre-existing pool that plays a significant role in the rate of acquisition. Moreover, they suggest that learning abilities at the early stages of training are linked to a decrease in cytosolic PKC activity.

495.21 WORKING AND REFERENCE MEMORY IN CBX RECOMBINANT INBRED MICE. D. E. Peeler*, Dept. of Neurosurgery, University of Mississippi Medical School, Jackson, MS 39216.

Two types of memory which have been described - reference and working - appear to function essentially independently. This implies not only separate CNS mechanisms, but also possibly different genetic influences. This was investigated in the CBX set of recombinant inbred (RI) strains. Association between genotype and performance in an 8-arm radial maze was assessed. A total of 100 male mice (N=100) from each of the 9 RI strains of the CBX set were familiarized with the maze for two consecutive days, 5 min per day, with all arms baited. For sessions 3-12, four arms per strain were baited. A dim lamp was placed on one corner of the maze, specific areas providing different shapes and textures as distant orientation cues. Each arm of the maze was dimly illuminated at its distal end. The mice were placed directly in the center of the maze. The distance of the maze, the type of bait used, and the location of the bait varied between sessions. Analyses utilized ANOVA and multiple range tests. Strains showed similar rates and levels of acquisition. There are significant differences among the strains for working memory (number of arm entries; number of baited arms entered before re-entry) and reference memory (number of non-baited arm entries), but the strain distribution patterns are different. Orientation change at the beginning of session 8 disrupted performance, presumably reflecting the contradiction of reference memory. The strain distribution pattern for reference memory indicated a possible major single gene effect. For some measures of working memory, the progenitor strains were statistically similar but the strain distribution pattern was different, implying some genetic interactions different from reference memory with no major single gene effect.

495.22 MOUSE STRAIN DIFFERENCES IN MORRIS WATER MAZE PLACE TASK PERFORMANCE. T. J. Patile*, Dept. of Psychology, New Mexico Tech., Socorro, NM, USA 87801.

AJ: DBA; and C57 mice were tested for acquisition and retention of place task in the Morris water maze. All animals were trained for 14 days (4 trials per day; 60 seconds maximum per trial), with the platform being moved left or right from the NE to the SE quadrant on day 8. AJ mice were unable to reliably find the platform, while the time taken by DBA animals in escaping the pool declined gradually during the 14 days of training, reaching the lighted corner significantly faster than either the DBA or C57 mice, while the DBA animals were significantly faster than the AJ mice in escaping the pool. Swim paths used by the 3 strains indicated that C57 mice selected the most efficient solution strategy for the task, while DBA animals did not acquire the same elegance in performance. AJ mice were never able to devise an appropriate strategy for solving the task. Neuroanatomical differences between the 3 strains are also described, and the implications discussed.


Behavioral, histochemical, neurochemical and electrophysiological evidence supports the hypothesis that the Naples High (NHE) and Low-Excitability (NEL) rat lines may be genetic model to study hippocampal functions (Cerezo et al., Neuropsychobiology 17:205-203, 1993). To further challenge this proposal, adult male rats of the NLE, NHE and random-bred controls (NRC) were tested in a Morris water maze. A video camera system was used to monitor and record rat behavior. In the place-learning task, one block of four trials per day was given for 5 days. Every day a rat had to find the hidden platform starting from all four locations to receive a reward. For the spatial discrimination task, the escape latency and the swim distance were measured. On day 6, the platform was removed from the pool and a 30-s trial was given. The time spent in each of the four quadrants was measured. The results indicate that the NLE/NHE rats, but not NRB-rats, decreased the time and the distance travelled to find the platform. During the acquisition phase, both NLE/NHE spent more time in the hidden platform, but the NLE/NHE rats were different from NRB-rats. The distance travelled was higher in the NHE than in the NLE/NRB rats. In the platform removal test, NLE/NHE rats spent less time in the hidden platform, but there was no difference on session 5, but were different from NRB-rats. These results indicate that both NLE/NHE rats are coherent with a non linear model of the inverted-U type with both extremes leading to impaired spatial processing. (Supported by CNR 92.0192.CT04 and 93.004682.CT04 grants).


The effects of exposure to spatial novelty on the expression of nitric oxide synthase were mapped in the brain of the Naples High (NHE) and Low-Excitability (NEL) rat lines by NADPH-diaphorase (NADP-D) histochemistry. Adult male rats of the NLE, NHE and control rats (NRC) were tested for 10 min in a 1-lit-maze and sacrificed at different time intervals thereafter (0, 2, 4, 10, 15, 30 min). The NADP-D activity remaining unchanged, but was negatively correlated with performance level. Since this result did not fit with the translocation hypothesis, this study was designed to examine the effects of an increased level of training on PKC activity in the hippocampus. Mice were trained in a spatial temporal discrimination task performed in an 8-arm radial maze for 2, 5, or 12 sessions. Then, PKC activity was measured in membrane and cytosolic compartments of the hippocampus. Behavioral results showed a slower rate of acquisition as compared to our previous studies. Biochemical results did show any significant decrease in cytosolic PKC activity for any group. Nevertheless, membrane bound PKC activity correlated significantly with the number of reference memory errors at the third day of training (r=0.74; p<0.001). Firstly, these data show that the decrease in cytosolic PKC activity is not cumulative across sessions. Secondly, they confirm the correlation between membrane-bound activity and performance accuracy. Further statistical analysis on all animals tested in our previous experiments (n=77), shows that the amplitude of the PKC cytosolic decrease is correlated with performance accuracy on the second day of training (r=0.24; p=0.02; one-tail).

Tackled together, these observations support the idea that the membrane-bound PKC activity derives from a pre-existing pool that plays a significant role in the rate of acquisition. Moreover, they suggest that learning abilities at the early stages of training are linked to a decrease in cytosolic PKC activity.

The widespread induction of NADP-D suggests that exposure to novelty activates overlapping neural networks across different organizational levels of the CNS, whereas the impaired expression in the NHE-rats provides support for studying the neural substrates of spatial and non spatial information processing. (Supported by CNR 92.0192.CT04, and MURST 40% grants).
496.1 GABAERGIC RETICULAR THALAMIC NEURONS PROJECT TO THE CONTRALATERAL MEDIODORSAL NUCLEUS OF THE CAT. L. Grütz*, M. Mariotti and M. Mancia. Institute of Human Physiology II, University of Milan, I-20133 Milan, Italy.

In order to investigate the topographical and chemical organization of the pathway involved in the regulation of the EEG synchronization at a thalamic level homerid neuronal peroxidase conjugated with wheat germ agglutinin (WGA--HRP) was injected into the intermediate part of the Mediodorsal nucleus (MD) of the thalamus. This approach was also combined with glutamic acid decarboxylase (GAD)-immunohistochemistry to provide additional criteria for the identification of retrograde labelled neurons. In cats under pentobarbital anesthesia WGA--HRP was stereotaxically injected into the intermediate part of MD. After a survival time of 24--48 hrs, the animals were perfused with the ascending acetic acid with mixed aldehydes, 25 µm frozen sections were horizontally cut. Alternate sections were reacted for WGA--HRP visualization using tetramethylbenzidine as a chromogen with cobalt intensification, and the peroxidase anti-peroxidase technique on immunohistochemistry for GAD. In the same sections GAD--immunohistochemistry, combined with the retrograde transport of WGA--HRP, revealed that the intermediate part of MD nucleus receives an important input from GABA synthesizing neurons located in the ipsi and contralateral rostral--pole of the RE nucleus. Although the distribution of GAD-immunoreactive retrogradely labelled neurons was similar both in the ipsi and contralateral areas the number was much smaller in the latter. The findings suggest that through both GABAergic ipsilateral and contralateral projections the RE contribute to a GABA mediated inhibition within the intermediate MD neurons population and to a thalamo--cortical synchronization.


We have examined brainstem neuronal activity in the primitive monotreme mammal, the echidna (Tachyglossus aculeatus) across the sleep cycle. While no periods of REM sleep were seen, unit activity patterns differed from those seen in placental mammals during nonREM sleep.

Twenty-two brainstem units were recorded from the midbrain and subcoeruleus region. Unit discharge rates were significantly lower in sleep than in quiet waking (F=3.8, p<0.05). However, both midbrain and pontine regions had substantially increased discharge variability (burst firing) throughout sleep (p<0.001).

Therefore, neuronal activity during sleep in the echidna does not resemble that seen in nonREM sleep in placental mammals. While the discharge rate of the neuronal population decreases, as in placental nonREM sleep, discharge variability increases, as in REM sleep. We hypothesize that both REM and nonREM evolved from this primitive "mixed" sleep state.

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Noradrenergic neurons of the nucleus locus coeruleus fire tonically throughout waking, decrease discharge in non-REM sleep, and cease discharging in REM sleep. Only serotonergic neurons of the raphe system and histaminergic neurons of the posterior hypothalamus display similar firing patterns. The locus coeruleus is densely innervated by GABAergic neurons. In vitro studies have shown that application of GABA onto noradrenergic neurons potently inhibits their activity. Based on this evidence, we hypothesized that GABA mediates the inhibition of noradrenergic locus coeruleus neurons during REM sleep.

GABA release in the locus coeruleus was measured as a function of sleep/wake state using microdialysis and HPLC techniques. Histological analysis confirmed the placement of two dialysis probes in the locus coeruleus and per-locus coeruleus areas. GABA release in the more caudal site was increased during both non-REM and REM sleep as compared with wake. Data from the more rostral locus coeruleus including the per locus-coeruleus area indicated a selective increase in GABA release during REM sleep as compared to wake and non-REM sleep. We have previously reported a similar selective increase of GABA release in the dorsal raphe nucleus during REM sleep (Soc. Neurosci. Abstr., 15:1815, 1993). These data suggest that GABA mediates the inhibition of noradrenergic locus coeruleus neurons during non-REM and REM sleep.

496.4 Simultaneous application of serotonin or carbachol facilitates NMDA-dependent bursts of firing in noradrenergic neurons in guinea-pig brain slices. P. Fort, A. Khateb, B.E. Jones and M. Mühlenbecket. Dept. of Physiology, CMU, 1211 Geneva 4, Switzerland and "Montreal Neurological Inst., McGill University, Canada H3A 2B4."

The nucleus basalus provides important cholinergic input towards the cortical areas. This study was designed to test whether the application of NMDA affects the discharge of the noradrenergic neurons. Our goal in this study was thus to identify the possible interactions between these substances. When NMDA was applied alone, it depolarized these cells and led them to fire tonically. However, when serotonin was added concomitantly, the neurons started to slightly hyperpolarize and then discharged rhythmically in bursts (21 out of 26 experiments). This effect persisted in TTX (n=4). Interestingly the duration of the TTS was greatly increased in presence of NMDA+ serotonin, as compared to the situation of NMDA alone (tested at different levels of membrane hyperpolarization). Almost a similar results were obtained for the simultaneous applications of NMDA and carbachol (9 out 19 experiments). These interactions might be the means by which cholinergic neurons could discharge rhythmically in bursts in vivo. (Swiss NSF, Canadian MRC, Fondation Fyssen and Lyonnais des Banques).


The nucleus basalis contains both cholinergic and non-cholinergic magnocellular neurones which respectively display distinct electrophysiological properties when recorded in guinea pig brain slices. Non-cholinergic neurones demonstrate high frequency (30-60 Hz) subthrehold oscillations and have the ability to discharge in clusters of action potentials firing at 2-6 Hz. In order to assess the potential modulation of their activity by GABA, the effect of muscimol, a selective GABAa agonist, was tested on the non-cholinergic cells. Out of 31 cells tested, 23 were depolarized by high concentrations of muscimol (10-4 M), yet concomitantly inhibited due to a marked decrease of their membrane resistance. With smaller concentrations of muscimol (10-6 M), tested on 8 cells, 5 were still depolarized, but their ability to discharge in clusters was greatly facilitated. This effect persisted throughout the duration of the agonist's application and was perfectly reversible. Furthermore, the effect was antagonized by bicuculline, a GABAa blocker. These results suggest that locally released GABA could serve to facilitate the cluster firing pattern of non-cholinergic neurones and thus the rhythmic activity of these cells could participate in the generation and/or maintenance of theta rhythms in the forebrain (Swiss NSF, Canadian MRC, Fondation Fyssen and Lyonnais des Banques).

496.6 HYPERPOLARIZING POTENTIALS IN MASSERETE MOTOEURONES ARE GLUCYLATED BY AN AUDITORY STARTLE REFLEX IN AUSTRALIAN RATS. CARBACHOL INJECTIONS IN THE NUCLEUS PONTIS DORSALIS. K.A. Kuhlmann, L. Lopez, J. Rodriguez*, R.H. Liu, E. Morales, M.I. Clark. Dept. of Physiology, Dept. of Anatomy and Cell Biology, and the Brain Research Institute. UCL School of Medicine, Los Angeles, CA 90024.

One striking phenomenon of active sleep is the occurrence of ponto-geniculo-occipital (PGO) waves. Merton and Bowker (Acta Neuro. Exp. 15:521-540, 1975) proposed that these waves are neuronal signatures of a startle response produced by internally generated acoustic stimuli. We have shown that virtually all motorneurones of the masseter muscle are accompanied by large amplitude PGP's recorded in lambar and trigeminal motorneurones (Polouritos et al., Somatosens. Motor Res., 5:109, 1991). We therefore decided to investigate whether startle responses induced by auditory stimulation are also accompanied by the activation of motorneurones. For this purpose we microiontophoretically injected carbamylcholine (200mM) into the nucleus pontis oralis of five o-chloroacetanilide anesthetized cats (160mg/kg i.v.) because carbamylcholine-induced muscle atonia is comparable to that which occurs during active sleep (Morales et al., J. Neurophysiol., 51:118, 1984). These results show that the response of the motoneurones to the injection of carbamylcholine is similar to that which occurs during active sleep (Morales et al., J. Neurophysiol., 51:118, 1984). Responses were quantified by measuring the duration of the neurons' discharge (duration+1.5ms) were recorded intracellularly (KC-liluted glass electrodes) from stereotically identified masseter motoneurones both before and after the injection of carbamylcholine. In 19 of the 20 masseter motoneurones recorded before the injection of carbamylcholine, the auditory stimulus evoked a discharge. In response to the injection of carbamylcholine, the auditory stimulus elicited a discharges in 9 neurones. The amplitude of the stimulus-induced hyperpolarizing potentials decreased and increased, respectively, suggesting that they are inhibitory post synaptic potentials. These findings indicate that the carbamylcholine-activated motor inhibitory system is responsive to a startle stimulus in the o-chloroacetanilide anesthetized rat and that the primary afferents with the production of a hyperpolarizing potential in masseter motoneurones. The physiological significance of this hyperpolarizing potential during active sleep may be in aiding in insuring the maintenance of muscle atonia that otherwise might be disturbed by auditory sensory stimuli. Supported by NS 23430 and NS 09999.
497.1
CIRCADIAN MODULATION OF THE RAT ACOUSTIC STARTLE REFLEX. P. W. Frankland and M.B. Ralph, Dept. of Psychology and Zoology, University of Toronto, Toronto, Canada MS 1A1.

The acoustic startle reflex (ASR) in rats exhibits robust circadian modulation, with ASR amplitudes greater in subjective day. Because the ASR is mediated by a circuit with few synapses, it provides a simple system to study the circadian modulation of behavior. We sought to identify the location of modulation by recording amplitudes of startle reactions evoked either acoustically or electrically via electrodes implanted in the primary startle circuit. Responses were measured at four hour intervals over 48 hours in constant dark conditions. Startle amplitudes were greater in subjective day for acoustically and electrically evoked responses from both the ventral lateral geniculate and medial longitudinal fasciculus. These results show that circadian modulation of startle occurs at some point in the circuit after the last brainstem visuex in the classic pontine reticular formation, at the level of spinal interneurons, motorneurons or at the neuromuscular junction.

In a second experiment, animals with cochlear lesions of the hypothalamic suprachiasmatic nucleus (SCN) exhibited no circadian variation in ASR amplitudes. Mean startle amplitudes were similar to pre lesion basal (subjective day) levels. These results suggest that circadian rhythm of ASR is driven by the SCN, and that the SCN may enhance startle amplitudes during subjective night.

(Supported by NSERC grant A7077 to J.S. Yoomans)

497.3
INDUCTION OF FOS PROTEIN IN THE RAT CIRCADIAN SYSTEM BY NON-PHOTIC STIMULI. L.掌心, K. Edler, B. Robinson and B. Woodside, Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Canada.

Immunohistochemistry of Fos proteins in the hypothalamic suprachiasmatic nucleus (SCN) and intergeniculate leaflet (IGL) of the lateral geniculate complex is often used as a tool in studies on the neural mechanisms and circadian processes involved in circadian rhythms of rodents. Photic stimuli that induce phase shifts of circadian rhythms in mice, rats and hamsters also induce the expression of Fos in the SCN and IGL. Photic stimuli that fail to induce phase shifts appear to be without effect on Fos expression in these structures. The effect of non-photic stimuli on Fos expression in the SCN and IGL is less well defined. Here we report that in rats, cortical injury (induced under pentobarbital anesthesia, 50 mg/kg, by lowering a 28 gauge needle 3 mm below the surface of the skull) or immobilization stress (60 min) induce the expression of Fos in the SCN and IGL. Stimuli such as i.p. saline (1 ml/kg) and cage transfer induce Fos expression in the IGL. Expression of Fos in the SCN and/or IGL in response to each of these stimuli is independent of the time of treatment, and is associated with expression in other brain regions, including many cortical, subcortical and hypothalamic areas such as the paraventricular nucleus, anterior hypothalamic nucleus and preoptic nucleus. SCN cells which express Fos in response to cortical injury or immobilization are located in subregions of the nucleus that also express Fos in response to photic stimuli. Results indicate that neural components of the rat circadian system respond to diverse types of non-photic stimuli and that Fos expression in SCN and IGL should not be regarded as light-specific.

497.5
CIRCADIAN RHYTHMS IN A GENETIC MODEL OF OBESEITY. B.E. Matthewson, H. L. Luman, and B.G. Nadkarni, Department of Psychology, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

The Zucker (fa/fa) rat is a widely used model of genetic obesity characterized by a variety of metabolic and endocrine abnormalities, and by low amplitudes of circadian phase advances. The nature of the circadian disturbance, and its possible role in obesity, are unclear. Body temperature rhythms (Tb) were recorded by telemetry in 7 obese and 5 lean Zucker rats under a 12:12 light-dark (LD) cycle and in constant dark (DD) and light (LL). Obese rats exhibited a phase advanced Tr in LD, the acrophase of the best fitting cosine function occurring 6.7 h earlier in comparison with lean rats (p <0.05). The amplitude of the cosine function did not differ between groups. The phase advance was retained in DD, indicating that it is not due to a resetting effect of light (14, 15). All rats exhibited free-running Trs with periods > 24 h (no group difference). This suggests that phase advances in LD are independent of pacemaker period and may reflect altered phase responses to light or changes in the sensitivity to photosensitive processes or coupling to slave oscillators. Obese rats also exhibited an advanced feeding rhythm, with more intake during the light period. The role of advanced rhythms in the development of obesity was examined by measuring body weight and food intake for 60 days in 2 additional fa/fa rats (6 weeks old); one group received ad lib food, the other only during the dark period, i.e., phase advanced feeding was prevented. After six days, the rats on ad lib food gained weight, but the nocturnal-fed rats exhibited a lower average daily weight gain (p <0.05), suggesting that circadian abnormalities may contribute to obesity.

497.6
PROGRESSIVE CIRCADIAN RHYTHM DECLINE IN ALZHEIMER'S DISEASE. William Fishtal, Veronique Fries and Barry Straus, Dept of Psychology, The City College & Graduate School, CUNY, New York 10011 and NYU Medical Center, Alzheimer's Disease Center, New York 10016.

This research is concerned with developing an early behavioral marker for AD. We have accumulated circadian rest-activity data (12 days per subject) from six healthy elderly subjects and fifteen very reliably diagnosed, community residing, AD patients (mild to severe), employing a miniature ambulatory clock and accelerometer.

The present report describes the detection and decline of the generator(s) of the circadian process by non-linear curve fitting, an iterative process that reduces noise and detects peaks that otherwise might be missed by manual determination of circadian rhythm data. Precise measures of amplitude, area, center and width of peaks, and overall area of a single circadian cycle are determined.

Typically in normal elderly (Gesell Development Score (GDS) > 1 Minis-Mental State (MMS) = 30), four activity peaks are detected accounting for 95% of the variance of the 24 hour circadian rhythm. Qualitatively, the circadian rhythm looks like a square wave with small perturbations throughout the 24 hour cycle. At night, subjects’ sleep periods are relatively free of waking movement activity.

In Alzheimer's Disease, advancing from mild to severe, (GDS = 4 to 6; MMS = 24 to 3) five to eight activity peaks are necessary to account for 92% to 94% of the variance of the 24 hour circadian rhythm. Most notable as dementia advances: (1) progressive increase in the number of peaks, (2) phase shift of peaks to earlier clock time, (3) increased variability, and (4) increased variance between peaks. The total amount of activity, though, remained relatively constant throughout the day.

Although the research is in an early stage, the progressive declines in the circadian data indicate that with decline in cognitive and functional competence, a concurrent and profound degradation of the circadian time keeping system occurs in Alzheimer's disease.
498.1
A circadian pacemaker located in the eyepath of Aplysia is responsible for generating a rhythm that synchronizes the circadian transcriptome. In the mouse, the czechetin is a potential circadian oscillator. In Aplysia, the czechetin levels oscillate with a period of 24 h, and they are regulated by light and circadian rhythms. In this study, we investigated the effects of phase-shifting treatments on the regulation of putative oscillator proteins (POPs) in Aplysia. The rhythmic expression of these proteins is modulated by light and circadian rhythms. To test this hypothesis, we used microarray techniques to study the expression of these proteins in Aplysia. The results showed that the expression of these proteins is regulated by light and circadian rhythms. These findings suggest that the czechetin is a potential circadian oscillator in Aplysia.

498.2
ESTROGEN RECEPTORS ARE NOT PRESENT IN THE CIRCADIAN SYSTEM OF THE OCTODONT DEGIUS, A DIURNAL RODENT. N. Goes, N. S. Douglas and T. M. Lee, Dept. of Psychology, University of Michigan, Ann Arbor, MI 48104, and Michigan State University, East Lansing, MI 48824.
Steroid hormones, in particular estrogen, influence circadian rhythms in various rodents, but the site of action for estrogen's effects is not yet known. Octodont degius are hibernating mammals that exhibit circadian temperature activity rhythms (Labyak, 1993). In the degu, elevated estrogen levels during estrus lead to phase advances in the wheel-running rhythm and increased levels of activity (Labyak and Lee, in press). Because lesions of the intracentral leaflet (IGL) of the thalamus in hamsters, a major afferent projection to the SCN, results in changes in the phase angle of activity onset, the IGL has been suggested as a candidate for estrogen's site of action. Estrogen receptors have been localized in the lateral geniculate nucleus (LGN), the primary circadian clock. The goal of this study was to identify the distribution of estrogen receptors throughout the degu brain. Three adult degu brains (one male, two female) were processed for immunohistochemical identification of estrogen receptors using the H222 antibody. ER-immunoreactive (IR) cells were found in the medial preoptic area (mPOA), bed nucleus of the stria terminalis (SNST), suprachiasmatic nucleus (SN), arcuate nucleus, paraventricular nucleus (PVN), infundibulum, ventral posterior medial nucleus (VMP), and amygdala. No ER-IR cells were found in the SCN or IGL. Thus, it appears that estrogen cannot directly influence the circadian system via the SCN or IGL in the degu.

498.3
EXPRESSION OF GROWTH HORMONE-RELEASING HORMONE (GHRH) AND SOMATOSTATIN (SRIF) GENES DURING REM SLEEP DEPRESSION IN THE RAT. T. Porraki-Heikonen, J. Toppila, M. Aisakkainen, F. W. Turek, D. Steenborg, University of Helsinki, Helsinki, Finland and Northwestern University, Evanston, IL 60208.
GHRH increases both SWS and REM sleep, while SRIF decreases sleep. Antiserum to GHRH decreases sleep and inhibits sleep rebound after deprivation. We hypothesized that REM sleep deprivation affects the expression of GHRH and SRIF genes. Male rats were deprived of REM sleep on small platforms for 24 or 72 h, and one group was allowed a rebound sleep of 24 h after 72 h of deprivation. Large platforms and animals taken directly from their cages served as controls to sleep deprived groups. In situ hybridization was performed on the paraventricular and arcuate nuclei using specific oligonucleotide probes for GHRH and SRIF. Numbers of cells expressing GHRH and SRIF were counted, as well as the area of silver grains over the labeled cells; serum GH was measured. Number of cells expressing GHRH in the arcuate nucleus was lower after 24 h of REM sleep deprivation than in control animals, and sleep was low during 72 h of deprivation. Numbers of cells expressing SRIF were elevated on small platforms after 24 h of deprivation but had returned to home control level at 72 h. Serum GH levels were decreased during the deprivation. We conclude that both elevated GHRH gene expression in the beginning of the deprivation and decreased GHRH gene expression through the deprivation may contribute to the low serum GH levels during the deprivation.

498.4
DIURNAL RHYTHMS OF MONOAFLINES AND THEIR METABOLITES IN CORTEX, HIPPOCAMPUS AND HYPOTHALAMUS OF MALE WISTAR RATS. D. Steenborg, M. Aisakkainen, J. Toppila and T. Porraki-Heikonen, University of Helsinki, Helsinki, Finland.
Normal values for the diurnal variation of monoamine and metabolite concentrations in brain tissue of male Wistar rats were needed for studies on effects of sleep deprivation. Rats aged 3 months and adapted to LD 12:12 (lights on 0600) were decapitated (rats with 2 h intervals for 24 h) and brain regions dissected, frozen, and subsequently analysed by HPLC-ED. Analysis of variance and cosinor analysis were used. 5-HT levels tended to decrease after onset of dark phase, while 5-HIAA decreased significantly after onset of light phase. SRIF levels were elevated after onset of dark phase. These changes suggest that the 5HT system is activated during the dark phase and inhibited during the light phase. These results provide new insights into the regulation of monoamine metabolism in the rat brain and may have implications for the treatment of depression.

498.5
CENTRAL THYROTOPIN-RELEASING HORMONE ADMINISTRATION PRODUCES PHASE SHIFTS IN THE CIRCADIAN RHYTHM OF HAMSTER WHEEL RUNNING BEHAVIOR. Keith A. Cary*, Patricia A. Soller, Neda Letew, Andrew Winokur, and Gary E. Pickard, Department of Psychiatry, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.
Several lines of evidence suggest that thyrotropin-releasing hormone (TRH) plays a primary role in the regulation of gonadal hormones. Analytic effects of this tripeptide have been established by numerous studies, and endogenous levels of TRH vary in response to increased and decreased CNS activity states. Modulation of arousal state would require integration and coordination of autonomic, neuroendocrine, and circadian functions. TRH and its receptors have been localized in the retina, suprachiasmatic nucleus (SCN), and pineal gland; monitoring the activity associated with circadian rhythm. This study evaluates the effects of TRH microinjection into the suprachiasmatic nucleus (SCN) of the hamster on 24 h wheel running behavior. The precision displayed by the hamster in wheel-running activity prompted our use of this species in these studies. TRH or saline was administered both by intracerebroventricular (icv) injection and site via chronic indwelling cannulae stereotactically placed in the SCN. Drug administration was performed at circadian times (CT) 6 and 18, times corresponding to subjective day and night, respectively. At CT 6, both phase advances and delays in wheel running activity were observed in hamsters receiving 10ngM and 100ngM TRH icv, whereas phase advances were observed following the same doses delivered directly in the SCN. The magnitude of the phase advance was dose dependent, with 10ng/M TRH administration being 35 and 50 minutes advance in wheel running activity, respectively. No changes in wheel running activity was observed following icv TRH administration at CT 18. These data support the hypothesis that TRH influences circadian rhythm.

Metformin, a biguanide hypoglycemic agent, was widely used for treatment of obese type 2 diabetic patients particularly because of its weight reducing effect. The hypothalamic neuropeptide Y (NPY) content was measured by radiomunassay and preproNPY mRNA expression was studied by quantitative in situ hybridization procedure in metformin treated (300 mg/kg orally for 12 days), in pair-fed and in ad libitum fed obes Zucker rats in order to elucidate possible mechanisms involved in the anorectic and body weight reducing effect of metformin treatment in genetically obese Zucker rats. The concentration of NPY in the hypothalamic paraventricular nucleus (PVN) was significantly higher in the metformin-treated and pair-fed rats than compared to the control animals. The expression of preproNPY mRNA in the arcuate nucleus (ARC) was similar in all three treatment groups. Both chronic metformin treatment and pair-feeding markedly lowered hypoinsulaemia in these animals. It is concluded that the treatment with metformin and pair-feeding, which result in comparable reductions in food intake, body weight gain and hypoinsulaemia, similarly increase NPY expression in PVN while not affecting preproNPY mRNA expression in the ARC. The increase in hypothalamic NPY content may be secondary to reduction of hypoinsulaemia during metformin treatment and pair-feeding. Thus, the therapeutic effect of chronic metformin treatment cannot be explained by changes in content or expression of hypothalamic NPY.

499.3 NALOXONE DECREASES BOTH CARBOHYDRATE AND FAT DIGESTION INDUCED BY NEUROPEPTIDE Y (NPY) A.S. Levine*, M.K. Grace, and C.J. Billington. VA Medical Center and University of Minnesota, Minneapolis, MN 55417

Central administration of NPY markedly enhances food intake in rats and appears to selectively stimulate intake of high carbohydrate diets. Naloxone has been reported to decrease intake of high fat (HF) diets much more effectively than high carbohydrate (HC) diets. In the current study we examined whether naloxone would block NPY-induced intake of HC and HF diets, given individually or concurrently. In our first study we presented rats with a choice of a high carbohydrate (HC) diet and a high fat (HF) diet and stimulated feeding by intracerebroventricular (ivc) injection of NPY. In this group of carbohydrate preferring rats (n=16), NPY induced HC intake (4.4 g/5 hr), but failed to increase HF intake (0.3 g/5 hr). Naloxone potently decreased HC intake of the HC diet at doses ranging from 0.1-3 mg/kg (1.7 g; 3 mg/kg; 2.5 g; 3 mg/kg; 1.7 g; 3 mg/kg; 1.5 g). In the second study the same rats were injected ivc with NPY and presented with either the HC diet or the HF diet (no choice). NPY stimulated both HC (3.9 g/5 hr) and HF (2.9 g/5 hr) intake, and naloxone decreased intake of both the HC (0.3 mg/kg; 1.9 g; 0.3 mg/kg; 1.2 g) and HF (0.3 mg/kg; 1 g; 0.3 mg/kg; 0.4 g) diets. Thus, we found that naloxone potently decreased NPY stimulated intake of HC and HF diets. This is in contrast to the published observation that naloxone has little or no effect on HC intake in food deprived rats offered a choice between HC and HF diets. Our data lend support to the idea that an NPY-opioidergic feeding system exists in the central nervous system.

499.4 DISOCIATIVE EFFECTS OF NEUROPEPTIDE Y ON FEEDING AND BODY TEMPERATURE AFTER INJECTION INTO DIFFERENT HYPOTHALAMIC SITES. P.J. Currie* and D.V. Coscia. Section of Biopsychology, Clarke Institute of Psychiatry, 250 College St., Toronto, ON, M5T 1R8 Canada

Recent research has shown the perifornical hypothalamus to be a particularly sensitive brain site in eliciting feeding after local injection of neuropeptide Y (NPY). Since other work has demonstrated that NPY injected into the paraventricular nucleus of the hypothalamus (PVN) exhibits both feeding-stimulatory and metabolic effects, the current study examined the simultaneous impact of this peptide on food intake and body temperature (Tb) of unrestrained rats following injection into the PVN, and contrasted it with similar measures after injection into the NPY-ergic lateral hypothalamus (LH). NPY (0-235 pmol) was infused in a volume of 0.4 µl using a 28-ga. microinjection assembly. Food intake and Tb were measured every 30 min for 3 h postinjection. Both PVN and LH injections of NPY increased food intake dose-dependently in the first 30 min test interval, with the PVN showing a stronger response than the PNv. In PVN treated rats, the increases eating was associated with a significant decline in T, which was also evident 30 min after NPY injection and lasted 2 h. A mean maximal T, decline of 0.97±0.20°C occurred within 90 min following PVN treatment of 235 pmol NPY. In contrast, NPY had no apparent effect on Tb when injected into the PFH. These findings support previous suggestions that while PVN NPY acts to stimulate a robust and relatively specific ingestive response, PVN NPY may participate in the integrative mechanisms responsible for the simultaneous regulation of feeding, heat conservation, and energy metabolism. (Supported by NSERC and INI of Canada).


Anorexia-cachexia-bearing (TB) rats exhibit a refractory feeding response to neuropeptide Y (NPY) and decreased hypothalamic NPY concentrations. Therefore, we hypothesized that constant hypothalamic infusion of NPY would correct the anorexia exhibited by these TB rats. Following anesthesia (100 mg/kg, ip, ketamine, 24 ga. infusion cannulae were implanted into the periifornical hypothalamic area of 32 male Fischer 344 rats, 18 days after sc. MCA stroke induced inoculation. These cannulae were connected to sc. Alzet minipumps (Model 2002) that infused NPY (125 mg/h) or artificial cerebrospinal fluid at 5 µl/hr). All food intake by both NPY-infused groups was increased immediately following surgery, 3 days later food intake in these groups was not different from that of the CSF-infused rats. Food intake by the TB rats continued to decline, with significant anorexia being noted 21 days after tumor inoculation. These results suggest a rapid desensitization of hypothalamic NPY receptors in both TB and control rats in the continuous presence of exogenous NPY.
499.7

Neuropeptide Y (NPY) is distributed into various regions of the hypothalamus. Inhibits robust eating responses. A recent magneto-mapping study employing nanoliter volume injections of NPY identified the medial perifornical area of the hypothalamus (PFR) at the level of the posterior hypothalamic nucleus and other areas as the most sensitive site for NPY's eating stimulatory effect (Stanley et al., Brain Res. 1993, 664, 304-317).

To identify the neural projections from this area, 30 nanoliters of the anterograde tracer, biotinylated dopamine amine (10%), was pressure injected into the mPFR of adult male Sprague-Dawley rats through glass micropipettes with 40-60 μm O.D. tips. Following survival times of 7-10 days, the animals were perfused, 465 nmol of biotin-dHPG was injected in avidin-HRP, and visualized with a nickel-iodinated diaminobenzidine reaction.

Dense projections of labelled fibers extended from the site of injection to the central gray, bed nucleus of the stria terminalis, medial and lateral preoptic areas, posterior hypothalamus, and median preoptic nucleus. Moderate projections were visible in the dorsal premammillary nucleus, parabrachial nucleus, lateral septum, paraventricular thalamic nucleus, precommissural nucleus, and locus coeruleus. Terminal labeling was also evident in the accumba, amygdala, lateral parabrachial nucleus, and nucleus tractus solitarius. Although the roles of these projections remains to be established, by acting on FFH neurons NPY could directly influence activity in multiple intran- and extrahypothalamic structures.

499.8
EFFECT OF FOOD DEPRIVATION ON NEUROPEPTIDE Y (NPY) MESSENGER RNA IN HYPOTHALAMUS OF SYRIAN AND DJANGARIAN HAMSTERS. J.G. Mercer, G. Selverston, G. Segalman*, J. Greenberg. The Rowett Research Institute, Buckburn, Aberdeen, U.K. AB2 9SB.

Imposed manipulations of energy balance that increase food intake in rats often have no effect or elicit an attenuated response in various hamster species. Thus, Syrian, Turkish and Siberian hamsters either fail to express, or exhibit only limited post-fast peak responses. NPY is unresponsive to glucocorticoid or inhibition of fatty acid oxidation (Bartness, Int. J. Obesity 1 suppl. 3:115, 1990). Syrian hamsters are, however, sensitive to the appetite-stimulating effect of Neuropeptide Y (NPY). Intracerebroventricular (Kulksosky et al., Peptides 9:1389, 1989). To assess whether endogenous NPY in hamsters is sensitive to alterations in energy balance we have examined the effect of food deprivation on preprodynorphin mRNA in the hypothalamus of two species of hamster, the Syrian hamster, Mesocricetus auratus, and the Djungarian hamster, Phodopus sungorus. PreproNPY mRNA was studied by in situ hybridisation using the cloned rat gene to synthesise antisense and sense 35S-riboprobes. Autoradiographic images were quantified by computerised image analysis, providing measures of integrated density (OD x mm²). In the Hooded Lister rat, which was employed as a control species, 48 h food deprivation induced a 2.24 fold increase in total hybridisation in the hypothalamic arcuate nucleus (ARC), relative to fed controls. The same manipulation resulted in a 1.5-fold increase in total hybridisation in the ARC of the Djungarian hamster. In contrast, preliminary studies of the Syrian hamster indicated that NPY expression in this species may not be regulated by food deprivation. This work was supported by SAOFD.

499.9
ALTERATIONS IN NUTRIENT METABOLISM HAVE DIFFERENTIAL EFFECTS ON GALANIN (GAL) AND NEUROPEPTIDE Y (NPY) IMMUNOREACTIVITY IN HYPOTHALAMIC NUCLEI. J. Wang, A. Akabayashi, C.T. B. Zafar, T. Silva, and S. E. LeWytzky. The Rockefeller University, New York, N.Y. 10021.

Hypothalamic injections of GAL and NPY have differential effects on nutrient ingestion. Food intake of NPY preferentially extracts individual sections of the hypothalamus and GAL preferentially stimulates fat intake. The question is whether endogenous production of these peptides in hypothalamic neurons is differentially responsive to signals related to the metabolism of these nutrients. To test this, two compounds that alter carbohydrate and fat metabolism were examined in albinor rats: 2-deoxy-D-glucose (2-DG, 200 mg/kg), which inhibits glucose utilization, and mercaptoacetate (MA, 600 μmoles/kg), an inhibitor of fatty acid oxidation. Intraperitoneal injection of 2-DG, compared to vehicle, caused a significant increase in NPY levels (measured via RIA), specifically in the paraventricular (PVN) and suprachiasmatic nuclei but not other areas. However, no change in GAL levels was detected. While MA, in contrast to 2-DG, produced no change in NPY levels, this metabolic inhibitor significantly reduced GAL levels, only in the lateral division of the PVN (~35%, p < 0.05). Since cAMP production is inversely related to intracellular glucose, the impact of ventricular injections of dibutyryl cAMP (25 μg) on NPY and GAL levels was also examined. This agent caused a significant increase in NPY levels specifically in the medial PVN and arcuate nucleus, while having no impact on GAL in any area. Thus, intracranial regulatory signals for GAL and NPY production appear to differ, consistent with the differential impact of these peptides on nutrient ingestion.

499.10

Cellular toxins (Tox, ricin A chain and monoclonal antibody) centrally injected with a monoclonal antibody (MAB) to a neuropeptide may specifically penetrate the peptideergic neurones and induce long-lasting disturbances of neuronal functions. Presently, the toxins were injected with a MAB to the Neuropeptide Y precursor molecule (MAB to C-Fankling peptide, C-PON) near the hypothalamic arcuate (ARC) or paraventricular (PVN) nuclei but, by itself, the injected C-PON-MAB was devoid of biological activity. The effects of one injection of C-PON-MAB or non specific IgG with toxins on spontaneous food intake and mRNA expression of NPY by in situ hybridization were studied.

In rats injected with the C-PON-MAB mixture into ARC, the daily food intake significantly decreased (26%, p<0.001) during the week after the injection. This was due to a decrease in the nocturnal food intake (30%, p<0.001) since the diurnal food intake was unchanged. On the day of injection, the diurnal food intake acutely increased (75%, p<0.001) whereas the following nocturnal food intake decreased (30%, p<0.01). The food intake was not modified by the injection of the specific mixture into ARC or PVN, and by the injection of C-PON-MAB/Tox. mixture into PVN. The injection of C-PON-MAB/Tox. mixture into ARC increased the mRNA expression of arcuate NPY which decreased after the same injection performed in PVN. The long-lasting decrease of food intake was due to an efficient immunological impairment of the arcuate NPY neurones in which the damages likely up-regulated the mRNA expression of NPY.

499.11
VAGOTOMY REDUCES FOOD INTAKE IN LEAN BUT NOT OBESE ZUCKER RATS. D. Greenberg*, D.L. Lewis, and A.J. Strohmeyer. Bourne Laboratory and Departments of Psychiatry and Neurology, Cornell University Medical College, White Plains, NY, 10605.

The genetically obese Zucker rat (fa/la) is hyperphagic compared to lean controls (F/7). This hyperphagia is characterized by increased meal size. The abdomen cavities have been implanted in the mice of satiety signals and severing the abdominal vagus reverses the hyperphagia of hypothalamic obesity (Powley & Oppenheim, 1974). We investigated the effects of total subdiaphragmatic vagotomy on the food intake and body weight of obese and lean male and female Zucker rats.

Male and female obese and lean Zucker rats received bilateral subdiaphragmatic vagotomies (by sham operations or each group). Rats were placed on a low fat diet (~ 1% fat) for 2 weeks and then placed on a high fat diet (30% fat) for an additional 2 weeks. Food intake and body weight was measured daily.

Vagotomy reduced the food Intake (28% p < .001) and body weight (23% p < .0003) of male and female lean rats in both diet conditions. Vagotomy failed to reduce the food intake of male or female obese rats fed the low fat diet, but did reduce the food intake (22% p < .05) and body weight (16% p < .001) of obese rats fed the high fat diet.

The fact that vagotomy reduced food intake in lean but not obese rats indicates that intact vagal function is required for the expression of hyperphagia and obesity in obese Zucker rats under these conditions.

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499.12
IVC ADMINISTRATION OF ANTI-GALANIN ANTISENSE Oligonucleotide: EFFECT ON FEEDING BEHAVIOR AND BODY WEIGHT. M.G. Hulsey*, C.G. McLeod, P. Fles and R.J. Martin, Department of Foods and Nutrition, University of Georgia, Athens, Georgia 30602

The neuropeptide Galanin has been reported to potentiate the consumption of high-fat diet in a macromolecular selection paradigm. This study investigated the effects of an IVC-administered antisense 2′-O phosphorothioate oligonucleotide, directed against the Galanin mRNA, on feeding behavior and body weight in rats. Sixteen male HSD rats were cannulated in the lateral vena cava, and cannula placement was confirmed using Angioiten II. Rats were given ad libitum access to tap water and a high-fat (60% w/w corn oil) semi-purified diet. Feeding behavior was recorded by computer. Rats were given a single injection of anti-Galanin oligonucleotide (5′-ctctgctctagctagctgac, N = 8) or a missense control oligo (5′-tggcgcctagctgag, N = 8) in the hour before the onset of the light phase (1200 h) and for consecutive days. Cumulative food intake was assessed at 12 and 24 h after each injection as well as over the six day injection period, and was subjected to one-way ANOVA. Body weight changes were also assessed, daily and subjected to one way ANOVA. Compared to missense controls, rats receiving the antisense oligonucleotide ate significantly more of the high fat diet (p<0.025) during the six day injection period. There was no effect on cumulative food intake at 12 h or 24 h after injection (p > .05). There was no effect on body weight change (p > .05). These data are incongruent with previous data from macromolecular selection paradigms where the peptide was administered, and may suggest that Galanin's role in the regulation of body energy balance may be less pivotal than previously thought.
499.13 MODULATION OF GENE EXPRESSION IN HYPOTHALAMUS OF OBESE ZUCKER (fa/xa) RATS IN RELATION TO AGE AND GENDER. M. Jhaver-Uniyat* and A. Burlet. The Rockefeller Univ. New York, New York 10021 and INSERM U308 Nancy, France.

Obesity in general predisposes Zucker rats to an autosomal recessive trait. Associated with this gene are various metabolic, endocrine and behavior disturbances, such as hyperinsulinemia, hyperthermia, enhanced lipogenesis, low energy expenditure, increased hypothalamic-pituitary axis, and hyperphagia. These phenotypic and metabolic abnormalities are observed relatively early in life of obese rats and are gender specific. Certain peptides and hormone receptors, by virtue of their early abnormal status in fa/xa rats or their role in primary metabolic systems show response to the changes associated with the obesity. This study examines the hypothalamic gene expression (mRNA, cRNA, mRNA) of certain carbohydrate-releasing factor (CRF) and glucocorticoid receptor (GR) by using the Northern blot analysis in male and female, lean and obese Zucker rats of 2, 5, and 9 wk old. The results demonstrate that: 1) higher gene expression of CRF in obese rats as compared to lean at 2, 5 and 9 wk age; 2) gene expression was significantly altered with age in both lean and obese rats; 3) gene expression of GR was significantly different in female vs. male, in lean and obese rats. Thus, the results of this study show that the gene expression for the CRF and GR is associated with development/maintenance of obesity.


Neuropeptide Y (NPY) induces feeding and has been associated with carbohydrate selection. 2-deoxyglucose (2-DG) blocks glucose utilization and it potentiates appetite for carbohydrates. Thus, blockade of glucose utilization could affect NPY gene expression in the arcuate nucleus (ARC) and NPY levels in the paraventricular nucleus (PVN). To test this, 2 studies were set up as follows: 1) 32 male rats were injected intraventricularly (ivc) with 18.3 μmol/10 g of 2-DG and 2) 30 male rats were injected i.p. 200 mg/kg of body weight. Both studies had a control (0.9% saline), a 2-DG with ad libitum food or 2-DG food deprived group. Two hours after treatment animals were sacrificed and NPY levels in the PVN and ARC NPY mRNA levels were determined using an NPY radioimmunoassay and an NPY cDNA probe respectively.

Table 1. Food, 2-DG PVN levels, ARC NPY mRNA levels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PVN levels</th>
<th>ARC NPY mRNA levels</th>
</tr>
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<tbody>
<tr>
<td>Saline ivc</td>
<td>0.7 ± 0.2</td>
<td>20.3 ± 2.4</td>
</tr>
<tr>
<td>2-DG/food ivc</td>
<td>2.9 ± 0.7</td>
<td>19.3 ± 1.5</td>
</tr>
<tr>
<td>2-DG/food ivc</td>
<td>19.7 ± 2.1</td>
<td>4.28 ± 0.25</td>
</tr>
<tr>
<td>Saline ip</td>
<td>15.4 ± 1.5</td>
<td>5.17 ± 0.28</td>
</tr>
<tr>
<td>2-DG/food ip</td>
<td>5.8 ± 0.5b</td>
<td>13.6 ± 2.0</td>
</tr>
<tr>
<td>2-DG/dep ip</td>
<td>13.4 ± 2.0</td>
<td>5.10 ± 0.29</td>
</tr>
</tbody>
</table>

2-DG increased food intake 4-fold, but did not affect NPY mRNA or peptide levels. These data suggest that 2-DG-induced feeding, through decrease glucose availability, does not involve changes in NPY activity.

INGESTIVE BEHAVIORS V


We have previously reported that intrathecally infused sucrose does not induce c-Fos like immunoreactivity (c-FLI) in the rat NTS unless a CTA has been formed to sucrose. Lesion studies indicate that brain areas rostral to the NTS are necessary for CTA acquisition and expression. Thus, we qualitatively surveyed c-FLI as a marker of neuronal activity in the pons and forebrain at the level of the hypothalamus 1 hour after treatment: intracerebroventricular infusion (5%), 6-20 min, LiCl alone (0.15 M, 125μg/kg, i.p.), intraperitoneal sucrose paired with LiCl, and rats treated with LiCl alone. The results suggest that c-FLI is a more sensitive measure of neuronal activity than the primary CTA formation and forebrain at the level of the hypothalamus 1 hour after treatment: intracerebroventricular infusion (5%), 6-20 min, LiCl alone (0.15 M, 125μg/kg, i.p.), intraperitoneal sucrose paired with LiCl, and rats treated with LiCl alone. The results suggest that c-FLI is a more sensitive measure of neuronal activity than cell-counting may reveal patterns of neuronal activity underlying CTA formation and expression.

Supported by the Matsers Foundation, the Whitall Foundation, and the Chairman’s Committee for Special Research.


Conditioned taste aversions can be produced by pairing a novel palatable substance with exposure to an aversive agent such as lithium chloride (LiCl). Using a rapid conditioning paradigm, the effects of LiCl pre-exposure on conditioned changes in taste reactivity responses were examined. Rats were pre-exposed to LiCl or NaCl (3.0 mol/kg, ip, NaCl-12 per group) and placed in an observation box for 30 sec with 30 sec intraoral infusions of 0.3 M sucrose administered at 5 min intervals. Both groups conditioned with LiCl produced fewer ingestive and more aversive responses than NaCl conditioned rats (p < .05). Comparison between the two LiCl-conditioned groups revealed that LiCl pre-exposure had no effect on ingestive responses and passive drip, but did reduce aversive responding relative to NaCl pre-exposed rats (p < .05). These results suggest that LiCl pre-exposure attenuates conditioning by selectively reducing aversive responses. (Supported by an NSERC to KPO).
500.3

**β-ESTRADIOL-INDUCED CONDITIONED SHIFTS IN SUCKROSE PALATABILITY IN MALE RATS**

K.P. Olsenknapp, J.Y. Ralls, and L.A. Eckel

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Conditioned taste aversions (CTA) can be produced by pairing a novel taste with exposure to a toxin. Previous studies have shown that estradiol decreases preference for saccharin in female rats. The present experiment examined the ability of estradiol to produce conditioned shifts in taste sensitivity responses to sucrose in male rats. Rats were subdivided into two groups and trained to associate sucrose solution, followed by s.c. injection of 17β-estradiol (100 μg/kg) or vehicle (polyethylene glycol). All rats were then given free access to water and 3 days later tested for their taste sensitivity to 0.3 M sucrose. The rats were placed in an observation box and three 30 sec intraoral infusions of sucrose were administered at 5 min intervals. The orofacial and somatic responses to the sucrose taste were videotaped to assess palatability. The estradiol group exhibited a significantly reduced ingestive behavior, and increased aversive behaviors (p<0.01), relative to the control group. When the two groups were subsequently given a 24 hr two-bottle choice test between sucrose and water, the estradiol group displayed a significant (p<0.01) CTA to the sucrose. Thus, estradiol-induced CTAs seem to be based on conditioned shifts in palatability.

500.5

**FOOD DEPRIVATION REDUCES STIMULUS DISCRIMINABILITY IN THE NUCLEUS TRACTUS SOLITARIUS OF THE RAT**

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The results of human psychophysical and animal behavioral studies suggest that a state of food deprivation alters hedonic perception of taste stimuli and broadens the range of substances an animal will accept as food. We recorded from the NTS of 72-hour deprived rats to determine whether these hedonic and behavioral changes resulted from alterations in the sensitivity of the taste system. The deprivation resulted in a 10% decrease in body weight at the time of recording. The evoked activity of 45 units from food deprived rats and 40 units from caloric replete rats to an array of 13 taste stimuli was recorded and analyzed. Food deprivation significantly reduced the spontaneous firing rate of NTS gustatory neurons and attenuates the response to the sodium and lithium salts. In addition, the breadth of tuning of salt-sensitive neurons was greater in food deprived rats than in controls. We then analyzed for differences in the code for taste quality. Activity profiles evoked by the salts (NaCl, L-lysine, and carbohydrates (Polysaccharide, glucose, fructose, sucrose)) across neurons were significantly more similar in deprived rats. Temporal patterns of activity in the firing rate of NTS neurons for the acids (HCl and citric) and sugars were also significantly more similar in deprived rats. The willingness of 72-hour food-deprived rats with a conditioned taste aversion to glucose to ingest an array of taste stimulus was tested. Results indicated a reduced ability in 72-hour food-deprived animals to discriminate between acid and sugar stimuli. The implication is that deprived rats are able to make fewer discriminations among tastes, an effect that could underlie their wider acceptance of potential foods.

Supported by grant DC03964 from the NIDDK.

500.7

**SIMULTANEOUS CONTRAST EFFECTS IN CHRONIC DECEREBRATED RATS**

P.S. Grange, M.D. Roitman, J.M. Kaplan, H.J. Grill, and R. Norgren

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Seven rats received full supraciliary deconstructions in two hemitranssections spaced one week apart. These rats, along with 7 control subjects, were then implanted with two intracranial cannulae and one anterior duodenal EMG electrode. On the first day following recovery from surgery, the rats received forty 10 sec infusions of 0.1 M sucrose. On a second day, they received identical infusions of 0.1 M sucrose. On two other days, the 2 concentrations were alternated for a total of forty infusions/day. All stimuli were delivered at a rate of 1.2 ml/min and the interstimulus interval was 30 sec. The rhythmic activity of the anterior duodenum, which closely follows gustatory licking, was evaluated during both the 10 sec infusion period and the 20 sec offset period. The results showed that during the offset period (1) both the intact and the decerebrate rats demonstrated a reliable concentration effect by generating more motor movements for the high than for the low concentration of sucrose. (2) While intact rats demonstrated contralateral effects, they failed to do so under these circumstances. (3) Regardless, chronic decerebrate rats did express simultaneous negative contrast. That is, they made fewer responses for the low concentration of sucrose when alternated with the high concentration than when presented alone (p< 0.004). Thus, an intact brain stem is sufficient for the occurrence of simultaneous negative contrast. Supported by DC-02216, DC-00240, MH-60553, DK-42284, and DK-21497.

500.8

**EFFECTS OF HEPATIC-CELIAC BRACH VAGOTOMY AND SITE OF POLYCOSE INFUSION ON CONDITIONED FLAVOR PREFERENCES IN RATS**

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We have shown (Horn & Mitchell, Soc. Neurosci. Abstr., 1993) that conditioned flavor preferences were produced using intragastric polyose infusion in rats in which hepatic-celiac branch vagotomy was blocked by hepatic branch vagotomy. The present study extended this research using a discrete trial procedure and a hepatic-celiac branch vagotomy. A normal control (n=7) and a hepatic-celiac vagotomized group (n=8) were both implanted with gastric fistulas and a duodenal group (n=8) was implanted with duodenal fistulas. While 22 hours food deprived, rats were trained for a total of 12 days. During training, rats received a flavor (CS+) paired with intragastric or intraduodenal infusion of 5 ml of 16% w/v polyose (P) and an flavored water (CS-) paired with intragastric or intraduodenal infusion of 5 ml distilled water. The flaves used were grape and cherry Kool-Aid. After training, rats were given two-bottle extinction preference testing for four days. Results showed that the combined hepatic-celiac vagotomized group failed to interfere with conditioned flavor preferences. Furthermore, flavor preferences appeared to be greater in the vagotomized animals. The results showed that polyose infused into the duodenum is as effective as intragastric infusion in conditioning preferences.
500.10


Intravenous intrusions of selective mu- and delta opioid subtype antagonists, naloxone and naltrexone, respectively, produced a high degree of naloxone (NTX) and the delta opioid antagonist beta-fumirexetine (BPNA) and the l-selective antagonist naltrexone (NTH) micromolar injected into MH to alter deprivation (24h) intake, 200 (500 mg/kg i.p.) hyperphagia, or sucrose intake. NTX was significantly reduced by NTX (10 mg: 208), BPNA (5 mg: 268) or NTH (5 mg: 315) in MH, 200 hyperphagia was significantly reduced by NTX (10 mg: 697), BPNA (20 mg: 83) or NTH (20 mg: 699 in MH only after 2h. Neither NTX, BPNA or NTH in MH altered sucrose intake. The NTX effects are compared with ventricular infusion to infer MH mediation of opioid antagonist actions on different forms of intake. Supported by NIDA DA 04194.

500.12

BLOCKADE OF DIETARY OBESITY DEVELOPMENT BY CENTRAL SELECTIVE OPIOID ANTAGONISTS IN RATS. J.L. Collet*, L. Leventhal, M. P. Parsons, and W.D. Cole* Dept. of Psychology and Neuropsychology, Special Program, Queens Coll., CONY, Flushing, NY 11367; Dept. of Nutrition, Memorial Sloan-Kettering Cancer Ctr., New York, NY 10021; Div. of Medicinal Chemistry, N.Y. Bethesdah, MD 20822.

Chronic injections of naloxone or naltrexone transiently reduce food intake and body weight, probably due to a limited duration of action. The reversible mu, delta, and kappa antagonists, reduced body weight and food intake over 2 weeks in adrenal rats, but failed to affect these variables in dietary-obese rats. Since acute administration of opioid antagonists reduce palatable intake, the present study examined whether body weight and intake of a high-fat, sweetened condensed milk and cheese would be altered by daily (11 days) central microinjections of l-selective mu (beta-fumirexetine: BPNA, 20 mg, mu, naloxonazine: NAS, 50 mg), kappa (norp-binalorphine: NPP, 20 mg, delta, DALCE, 40 mg) or delta, (naltrindole 5'-isothiocyanate: NTII, 20 mg) opioid subtype antagonists. Rats exposed to the palatable diet gained significantly more weight (+98 vs +30) than chow-fed controls. Significant reductions in body weight occurred immediately and progressed thereafter for each antagonist: BPNA (-5%), NAS (-1%), NTH (-5%), DALCE (-7%) and NTII (-7%). Corresponding decreases in milk, fat and chow intakes were also observed following antagonists treatments. These data implicate each opioid receptor subtype in maintenance of body weight under caloric conditions. Supported by NIDA DA 04194.

500.14

EEG RESPONSES TO ICE CREAM AND PAIN: OPIOID AND PREFERENCE EFFECTS. D.D. Kroeh*, B.A. Comell, R.J. Davidson, J. Williams, L. Redmond, L. Vilas, University of WI Dept of Psychiatry, Madison, WI 53792.

Research by our group and others has demonstrated that (1) treatment with opioid agonists increases and treatment with opioid antagonists decreases intake of and preferences for highly preferred foods and fluids in animals and humans; (2) intake of highly palatable foods affects the endogenous opioid system; and (3) opioid receptors are involved in the rewarding effects of palatable stimuli; and (3) states associated with positive affect and approach behaviors are associated with release of the left frontal central hemispheres (R4R) as measured by EEG in humans. In order to determine whether the eating of a highly preferred food resulted in altered response to pain, changes in mood, and R4R, due to effects on the endogenous opioid system, we are conducting an experiment in which we have responses of young female subjects (classified as ice cream like or non-like) on the basis of their responses to a questionnaire to ingesting ice cream (750 cal in 10 minutes) followed by either naloxone or saline are measured. The same responses are also measured in humans and non-humans during a no eating, saline trial. Initial results in three ice cream like and non-like trials are available; additional data are being collected. After ice cream ingestion, naloxone treatment resulted in a large shift to relative right activation on the EEG in like; this effect was not seen in non-likes. Also, the three likes showed a relative increase in right-sided EEG activation on the ice cream trial, in contrast to the non-likes, which showed a relative decrease in right-sided EEG activation. This preliminary data support the hypothesis that subjects who highly prefer ice cream differ from those subjects who are less effective and analytic responses to palatable food ingestion. This research was supported by NIDA grant DA05471.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
500.15 NITROUS OXIDE STIMULATES FOOD INTAKE IN NON-DEPRIVED RATS. J. M. Rodby and C. L. Malow, Department of Psychology, Marquette University, Milwaukee, WI 53233.

Previous research suggests that nitrous oxide (N₂O) exerts one or more of its effects through a benzodiazepine (BZP) receptor system. Further, BZPs are known to influence ingestive behaviors in several animal species. This study examines a possible N₂O effect on food intake in an unrestrained rat.

In exp. 1, male Long-Evans hooded rats were exposed to one of four concentrations of a N₂O and O₂ mixture (10-40% N₂O) or to room air circulated through an enclosed environment. Following an initial acclimation to gases, ground chow and tap water were provided ad lib in the same environment for a 60 min test. Intakes at 30 and 60 min were corrected for baseline weight gain and body weight (BWT) and in other exprs. with ANOVA procedures and, as appropriate, Dunnet's or adjusted Student-t tests; alpha level was set at P<0.05. Food intake increased in a dose-related manner with increasing concentration of N₂O, differing significantly from room air controls at 20% N₂O.

In exp. 2, pretreatment with 10 or 20 mg/kg (p) of the BZP receptor blocker, flumazenil, failed to attenuate the hyperphagia induced by a challenge concentration (20%) of N₂O.

In exp. 3, pretreatment with the opioid antagonist, naloxone (0.1-10.0 mg/kg, sc), attenuated 30% N₂O-induced hyperphagia in a dose-related manner; all dosages were significant relative to vehicle control. These data fail to support an hypothesis that N₂O-induced hyperphagia in the rat is mediated by a BZP receptor. The data do, however, implicate an opioid mechanism.


Although innervation of the hepatic parenchyma has been demonstrated in many species, its existence in the rat has been controversial. The intralobular innervation of human liver has been characterized using immunocytochemical staining for a variety of neural markers. To determine whether the hepatic parenchyma of rats is innervated, we examined liver tissue immunohistochemically for the presence of neurofilament protein 200 (NF 200) using a monoclonal antibody to NF 200. Reaction product indicative of fine neuronal processes was observed in close association with hepatocytes in certain areas of the liver. The processes were seen transversing several hepatocytes and often had a beaded appearance indicative of varicosities. NF 200-like immunoreactivity was also found in association with the hepatic vasculature suggesting a vascular innervation as well. Western blot analysis of liver tissue confirmed the presence of NF 200. These observations provide evidence for intralobular innervation of the rat liver.

500.17 HEPATIC PORTAL AND VENA CAVA GLUCOSE AND AMINO ACID INFUSIONS DECREASE DAILY FOOD INTAKE IN RATS. A. E. Willis, D. A. Walls and H. S. Koopman. Dept. of Medical Physiology, Univ. of Calgary, Calgary, Canada, T2N 4N1.

Intravenous infusions of glucose and/or amino acids into the vena cava cause daily food intake to decrease by 50% to 100% of the kilocalories (kcal) infused (Walls & Koopmans, 1992). Since glucose and amino acids are normally absorbed from the gut into portal circulation before they are taken up or metabolized in the liver or passed on to the rest of the body, we examined whether glucose and amino acids infusions into the hepatic portal vein (HP) of male Lewis rats would lead to better caloric compensation than vena cava (VC) administration. A 10 kcal glucose infusion decreased daily food intake to a similar degree in the HP (3.3 ± 1.5 kcal) and VC groups (2.5 ± 4.9 kcal, p > .90). Daily food intake was significantly decreased (p < .01) during the 20 kcal infusion by 12.8 ± 4.8 (HP) and 14.7 ± 3.1 kcal (VC). There was no significant difference between the two routes of infusion (p > .75). With the 20 kcal amino acid infusion food intake was significantly reduced (p < .005) by 17.4 ± 4.9 kcal in the HP group and 17.7 ± 2.7 kcal in the VC group. Again there was no significant difference in route of infusion (p > .90).

There was no significant effect of treatment or route of infusion on the rate of body weight gain. These results show once again that infused nutrients can provide part of a signal that controls daily food intake. Furthermore, the liver is not likely to be the site where this signal is generated even though it plays a central role in nutrient metabolism. The infusion of glucose or amino acids into the portal vein would generate high hepatic plasma values of these nutrients but produced reductions in daily food intake that were similar to those found after vena cava infusion.

501.1 DIFFERENTIAL MATING STIMULATION EFFECTS FOS EXPRESSION IN BRAIN AND SPINAL CORD OF THE FEMALE RAT. R. Komberg, J. W. Lee and M.S. Emdin. Department of Biology, Boston University, Boston, MA 02215 USA.

Threshold amounts of cervical stimulation (CS) received during mating are essential for the initiation of the prolactin (PRL) surge of early pregnancy in the rat. The expression of FOS in brain and spinal cord in response to amounts of CS which are sufficient or insufficient to induce PRL surges was examined. Ovariectomized rats primed with estrogen (EB, 40ug/kg) and progesterone (2mg/kg) received subthreshold (mouths-without-innission; MO), peristimulus (5 or 10 innasions, I), supratreshold (15 I) mating stimulation from males or were taken directly from the home cage (HC). Animals were sacrificed 1 hr post mating, and brain and spinal cord sections were stained for FOS protein using standard techniques. Numbers of FOS-immunoreactive (FOS-IR) cells increased in proportion to the number of I in the medial amygdala (mAeMY) and bed nucleus of the stria terminals (BSTN) and in response to 1 compared to MO and HC in the praeoptic area (POA) and ventromedial nucleus of the hypothalamus (VMN). The L6 segment of the cord showed graded numbers of FOS-IR cells following 5 or 15 I; this effect was restricted to laminae I and X. At thoracic (T12), lumbar (L1-L3) and sacral (S1-S2) levels, stimulation did not increase FOS above HC levels. MO animals at all spinal levels examined had significantly higher FOS-IR than HC and I animals, suggesting an analgesic effect of CS on neural activity in the cord. Since the mAeMY, BSTN and L6 showed graded responses to CS, these areas may be involved in acquisition of information relevant to initiation of PRL surges. Supported by HD21802 to M. S.

501.2 ALTERNATIVE MALE TYPES IN TREE LIZARDS HAVE DIFFERENT DELAYED STEROID HORMONE RESPONSES TO AGONISTIC ENCOUNTERS. R. Knappe and M.C. Moore. Dept. of Zoology, Arizona State Univ., Tempe, AZ 85287-1501.

Steroid hormones are known to mediate agonistic behavior in a variety of species. We are examining the role of testosterone (T) and corticosterone (B) in agonistic behavior in a species where a male can be one of two permanent types with respect to aggressiveness. Previously, we reported finding a delayed (1-day) increase in plasma B levels, but no changes in T, in aggressive-morph male tree lizards (Urosaurus ornatus) who won short contests in the laboratory (Amr. Zool., 320(24A), 1992). We have now repeated the experiment to determine whether this delayed effect of winning occurs under more natural conditions, and if it also occurs in the less aggressive morph. Staged encounters were conducted using a male presented to marked lizards in the field. The two types of males differed in their hormonal response to the encounter. Winning staged encounters had no significant effect on plasma T or B levels in the territorial, more aggressive morph one day following the encounter. However, males of the less aggressive, satellite/nomadic morph exhibited decreased T (ANOVA, p = 0.02) and increased B levels (p = 0.003) relative to controls. A contributor to morph-specific levels of aggression documented earlier (Horn Behav., 26:56B, 1992) may be a morph-specific response of T and B levels following male-male encounters. However, morph differences in aggressiveness may be mediated by differential sensitivity of plasma T to increases in B, as i.p. injections of 460 ng B revealed no difference in T levels for the two types of males. The results do, however, suggest a hormonal mechanism whereby frequency of male-male encounters may influence whether a male of the less aggressive morph is a satellite or nomadic.
10.5 LHRR MICROINJECTED INTO THE MEDIAL PREOPTIC NUCLEUS (MPOA) INHIBIT GONADIALLY DEFEMINIZED MALE RATS. J.G. Bloch, J.G. Kohlert, P. Butler, M. Stowell, R. Huber, R. Johnson, C. Beraison, B. Krasner, B. Walden, A. Fleming, Psychology Dept., BYU, Provo, UT 84602. Gelatin (GAL) microinjected into the MPOA facilitates male-typic behavior in the rat (Bloch et al., Physiol Behav 54, 93). GAL stimulates LHRR and is found within LHRR cells located within the medial preoptic area. These animals have reported that subcutaneous LHRR facilitates male sexual behavior. I.e., LHRR also stimulates testosterone but this result is not site-specific. No effect was noted with LHRR in the MPOA, but gonadally intact males behave maximally and thus may be insufficiently determined the behavioral response of 3IU saline (SAL) or 20,50,100,500UG LHRR microinjected into the MPOA of gonadectomized male rats implanted with 2mg testosterone-filled Silastic capsules; these produce low levels of behavior, allowing for more or in combination with P. Low levels of LHRR in the male do not therefore result from a suppression by gonadal steroids of LHRR neurons mediating the lordosis-stimulating effects of CCK. (NIMH HD27334)

10.6 INHIBITION OF TESTOSTERONE AROMATIZATION IN THE INTACT MALE RAT PREPARED OR RECEPTIVE ESTROGEN ENHANCES ESTROGEN INSENSITIVITY AND IMMUNOREACTIVITY AND REDUCES MATING. A. N. C. Clancy, D. W. Zampa, and R. P. Michael. Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, College of Allied Health, Atlanta, GA 30322. 

C dopulatory behavior was studied in 5 groups of sexually experienced, intact male rats in which: (i) the nonsteroidal aromatase inhibitor Fadrozole (Ciba-Geigy CGS 16949A) was delivered bilaterally into the medial preoptic area (POM) together with normal saline given i.c.v. in (i) and (ii) normal saline was delivered bilaterally into POM together with the same Fadrozole dose given i.c.v., (ii) Fadrozole was delivered bilaterally into the medial preoptic area with saline given i.c.v., (iii) Fadrozole was delivered bilaterally into the lateral preoptic area together with saline given s.c., (iv) Fadrozole was delivered bilaterally into the lateral preoptic area together with saline given s.c. and (v) unoperated controls. Mounting and ejaculation were significantly decreased in rats receiving Fadrozole in POM compared with the behavior of rats in the other 4 groups. Few differences occurred between rats in the latter 4 groups, all of which continued to mate. The H222 (Abbot Laboratories) and ER-715-anti-estrogen receptor (ER) antibodies were used to examine the distributions of ER immunoreactive (ERa) neurons in gonadectomized controls and some of the groups in i, ii, and iii. Since of ERa neurons in the anterior hypothalamus was abolished in the estrogen-deficient rats. In summary, our results indicate that the presence of estrogen is important for copulatory behavior in male rats and that H222 ERa can be used to identify the neurons in POM affected by Fadrozole. (Funded by USPHS grant MH 39506 and the Emory University Research Committee).

10.7 DIFFERENCES IN ESTROGEN SENSITIVITY IN WHITFALL LIZARDS L. Young and D. Crews. Dept. of Zoology, University of Texas, Austin TX 78712. 

C. unipares is a unisexual species of whiptail lizard whereas C. inornatus is a sexual species and the maternal ancestor of C. unipares. Together they represent an excellent model for investigating the evolution of hormone-brain-behavior relationships. Plasma levels of estradiol in C. unipares are approximately 5-fold lower than those of female C. inornatus in the same state. This translates into species differences in dose-response curves for estradiol benzoate (EB) induced female sexual behavior (receptivity) (p<0.01). Ten-fold smaller dosages of EB were used for stimulation of behavior in C. unipares compared to female C. inornatus. In situ hybridization analysis indicates that estrogen induction of progesterone receptor (PR) expression in the ventromedial hypothalamus (VVM) also differs between the species. EB was significantly more potent in ovarciotomized C. unipares than in ovarciotomized female C. inornatus at inducing PR gene expression within the VVM (p<0.01). Preliminary results indicate that species difference in estrogen receptor expression in the VMH may account for the differences in behavioral and molecular sensitivities to EB. These results indicate that differences in behavioral sensitivity to estrogen lies in the estrogen target neurons in the brain area (VMM) controlling receptive behavior. Furthermore, these differences in sensitivity may be involved in the evolution of species differences in circulating hormone levels and of hormone sensitivity of steroid dependent behaviors in general. Supported by NSF Pre-doctoral Fellowship (LJY) and NIMH 41770 (DC).
501.9
FOS PATTERNS IN MALE HAMSTER VOMERONASAL PATHWAYS: PHEROMONE STIMULATION AND EFFECT OF EXPERIENCE. Gwen Fernandez-Powell* and Michael Meredith Neuroscience Program, Florida State University, Tallahassee, FL 32306.

Removal of vomeronasal organs (VNX) from sexually naive, but not from sexually experienced male hamsters results in severe deficits in mating behavior.

Previous studies of VNX and intact males indicate a preferential activation of VN pathways (Accessory olfactory bulb, Medial amygdala, Me) during mating behavior and pheromonal stimulation. Activation in the medial preoptic area (MPOA) mainly reflected copulatory performance. Here, we used c-fos expression to analyze patterns of neural activity in sexually experienced intact and VNX males exposed to pheromonal stimulation either alone or during mating. Males from each group were placed in clean boxes with fresh bedding and each exposed to female hamster vaginal fluid (HVF) or a sexually receptive female for 45 mins. An additional 15 mins were perfused with 4% paraformaldehyde. Control animals were put in clean boxes with fresh bedding and perfused 90 mins later. Fifty um vibratome sections were processed for immunocytochemistry using a polyclonal Fos antibody. (Cambridge Research). When exposed to HVF, experienced males had higher levels of Fos expression in the MPOA than did inexperienced males, whether intact or VNX. Mated animals all had extensive Fos expression in MPOA but here there appeared to be a denser expression in intact than VNX animals. Fos expression in M, among HVF exposed males, was highest in intact experienced animals with lower levels in inexperienced animals and in VNX animals. Mated animals had a higher overall level but similar relative levels of expression.

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501.11
ESTROUS FEMALE ELICITS DOPAMINE RELEASE IN MPOA OF INTACT, BUT NOT CASTRATED, MALE RATS. J. Du, D. S. Lorrain, L. Matsevich, and E. M. Hull*. Department of Psychology, SUNY at Buffalo, Buffalo, NY 14260.

Extracellular levels of dopamine (DA) metabolites DOPAC and HVA increased in the medio preoptic area (MPOA) of male rats during copulation; DA was below the sensitivity of the assay (Hull et al., 1986). The presence of an estrous female increased the catecholamine amperometric signal in MPOA, but the contribution of DA could not be determined (Blackburn & Pfus, 1992). This experiment tested whether extracellular DA, serotonin (5-HT) and their metabolites in the MPOA of male rats were affected by exposure to an estrous female behind a barrier or by copulation. We also tested if testosterone (T) facilitates the DA response to the female. Microdialysis samples were assayed using an LC Packings capillary column and Antec electrochemical detector. In intact males, DA, DOPAC and HVA increased in the presence of the female and during copulation; 5-HT and 5-HIAA were not affected. In castrates with T replacement DOPAC and HVA increased significantly in the presence of the female and during copulation; DA increased slightly. In castrates without T, DA fell throughout the experiment, and DOPAC and HVA did not rise in the presence of the female; these animals did not copulate. Since DA in MPOA enhances male sex behavior (Hull et al., 1986), T may influence copulation in part by increasing DA release in MPOA in response to an estrous female. (NIMH grant #40826 to EMH)

501.13
AXOTOMY AND TESTOSTERONE SENSITIVITY OF THE RAT BULBOSPONGIOSUS MOTOR SYSTEM. W.F. Collins III, W. Ha, and M. Gonzalez. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

Rat bulbospinosus (BS) motoneurons exhibit androgen dependence in adulthood. However, it is not clear whether androgens act directly on BS motoneurons or indirectly via the BS muscle. To address this question, we have examined the effect of axotomy on the androgen sensitivity of the rat BS motor system.

The BS muscle was sectioned unilaterally in 180 gm males anesthetized with ketamine and xylazine. The rats were then castrated (with or without testosterone pellets implants) or sham-castrated (sham). After 28 days survival, the rats were sacrificed, and the left and right BS muscles were removed and weighed. Transverse sections from L4-L5 spinal cords were counterstained with cresyl violet, and the somal cross sectional areas of 20 randomly selected motoneurons in both the left and right dorsomedial nuclei from each rat were measured. The data were analyzed using ANOVAs.

As expected, castration produced decreases in mean BS muscle weight and motoneuron somal cross sectional area (73% and 178% of sham controls; respectively) on the axotomized and control side. Chronic denervation of the BS muscle resulted in a 53% decrease in weight (compared to contralateral control), and the BS muscle exhibited a further loss in weight (59% decrease compared to denervated sham) following castration. Chronic axotomy produced a 23% decrease in BS motoneuron somal cross sectional area (compared to contralateral control). Furthermore, somal cross sectional area of axotomized BS motoneurons was 12% smaller in castrated rats compared to sham controls. All castration effects were reversed by testosterone administration and are significant at p<0.01.

Thus, both denervated BS muscles and axotomized BS motoneurons are sensitive to androgen. However, the effect of castration on axotomized BS motoneurons is attenuated compared to that observed in axotomized intact BS motoneurons.

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501.10
STERIOD-INDUCED REPRODUCTIVE BEHAVIOR IN OBESE ZUCKER FEMALE RATS. C.L.M. Bezerra* and D.H. Olds. Department of Psychology & Neuroscience Research Institute, University of California, Santa Barbara, CA 93106.

Obeze Zucker female rats are infertile. Anecdotal evidence suggests ovary-intact obese Zucker females display less reproductive behavior than their lean counterparts. The present study was designed to compare reproductive behavior induced by exogenous steroid hormones in ovariectomized (OVX) lean and obese Zucker rats. OVX animals were given estradiol benzoate (EB; 15-100 μg/kg) or EB plus progesterone (P; 2-10 mg/kg), and tested for sexual receptivity. At the highest EB dose obese Zucker females displayed lordosis less frequently than lean rats (Lordosis Quotient, LQ = 9 ± 6% vs. 32 ± 13%, respectively, p<0.01). At the lowest doses of EB plus P lean females were maximally responsive (LQ = 93 ± 4%); Zucker obese females, in contrast, were only slightly receptive (LQ = 26 ± 11%; p<0.0001). Increasing the dose of either EB or P, administered in combination with the lowest dose of the other hormone, produced responses in obese Zucker females that were comparable to those observed in lean rats. These data suggest that considerably higher doses of EB and/or P are required to elicit robust lordosis responses in O VX obese Zucker, as compared to lean rats. This behavioral hyperresponsiveness may contribute to infertility in the obese Zucker female rat. (This work was supported by NIH HD 21483 and the American Psychological Association.)

501.12
SEXUAL EXPERIENCE INCREASES COPULATION INDUCED FOS-LIKE IMMUNOREACTIVITY IN THE MPOA. L.A. Llamas* and E.M. Hull. Department of Psychology, SUNY at Buffalo, Buffalo, NY 14260.

Male sexual behavior increases the induction of the immediate early gene c-fos in the medial preoptic area (MPOA) (Robertson et al., 1991), a brain area important in the regulation of male sex behavior. We recently reported that a DI antagonist lowered the number of copulation induced Fos-like immunoreactive cells in the MPOA (SN, 1993). Male rats improve their copulatory efficiency with sexual experience. We hypothesized that sexual experience may affect copulation induced Fos-like immunoreactivity in sexually experienced relative to sexually inexperienced male rats. One group of male rats received sexual experience every five days for seven weeks, at which time they were able to achieve three ejaculations in a 30 minute interval. Another group of male rats of equal age received no sexual experience prior to the test day. On the test day, all males were allowed to achieve one ejaculation, and brains were subsequently assayed for Fos-like immunoreactivity. A nonopercutaneous control group was also included. After one ejaculation, an increase in Fos-like immunoreactivity in the MPOA was observed in sexually experienced relative to sexually inexperienced rats. Since dopamine in the MPOA is released during copulation and is important in the regulation of male sexual behavior, increased Fos-like immunoreactivity may reflect a more sensitive dopamine system in sexually experienced male rats. Supported by NIMH grant #MH48286 to EMH.
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05.3


Niacotine facilitates human attention, memory and sensoromotor function. A visual spatial working memory (VSWM) task that utilizes these functions activates several frontal and parietal cortical regions as measured by functional magnetic resonance imaging (FMRI). Such evidence exists for the involvement of forebrain dopamine (DA) systems in VSWM as evidenced by the effects of nicotine on these systems, without the effects of nicotine on task-induced FMRI activation. Single shot echo planar imaging was obtained on a 1.5 Telsa GE Sigma scanner using an insonated, balanced, torque 3-axis head coil designed for random gradient switching. To obtain images throughout the entire brain volume, a shielded quadrature elliptical copped transmit/receive birdcage radio frequency coil was used. Four experienced smokers performed the VSWM task while FMRI data were acquired (TR=5 sec; TE=40 msec; 8 mm slice thickness). A total of 7 cycles of task repetition (30 sec rest, 45 sec task). Nicotine (0.75, 1.5 or 3.0 mg) or saline was injected iv over a 1.3 sec period during the task cycle. The most common nicotine effect observed (defined as appearing in 4 contiguous pixels) was an increase in the magnitude of the task-induced activation response in both frontal and parietal regions with doses as low as 0.75 mg. In each case, an increase in activation magnitude was seen within 1 minute of drug (but not saline) administration. The average injection-induced increase in signal was 3.9% after saline, 3.5% after the low dose (0.75 mg) nicotine dose, and 8.6% after the median (1.5 mg) nicotine injection. The present data suggest that nicotine enhances regional neuronal activation induced when performing a VSWM task.

05.4


Nucleus accumbens septi (NAS) is identified as a critical structure for the habit-forming actions of opioids, and glutamatergic afferents from the frontal cortex have been suggested to regulate NAS activity. Accordingly, the present study was designed to determine fluctuations in NAS glutamate levels during intravenous heroin self-administration. Rats with NAS dialysis probes were allowed to self-administer heroin. Extracellular fluid from NAS was sampled at 5min intervals by micro-dialysis and was assayed using HPLC with electrochemical detection of OPA amino acid derivatives. Glutamate concentrations in the dialysate increased during heroin self-administration stabilizing at 200% to 400% above baseline. Glutamate concentrations appeared to increase further during extinction conditions that followed the self-administration period. The glutamate elevation during self-administration coincides with reported elevations in dopamine; the elevation in glutamate during extinction occurs at a time when dopamine levels are falling. Thus behavioral states appear capable of dissociating fluctuations in extracellular levels of the two transmitters, suggesting a more complex glutamate-dopamine interaction than has been recently hypothesized.

05.5

IBOGAINE ANTAGONISM OF MORPHINE-INDUCED HYPERACTIVITY: ENHANCEMENT BY PRIOR MORPHINE EXPOSURE AND ROLE OF KAPPA OPIOID RECEPTORS M.G. Steckler, J.A. de la Fuente, and D.G. Sig, Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208.

Ibogaine, an indole alkaloid, is currently being investigated as a potential anti-addictive therapy. It has been shown to decrease morphine self-administration and to inhibit both morphine-induced locomotor stimulation and morphine-induced dopamine release in the nucleus accumbens and septum, in both rats and humans, the effects of ibogaine appear to be quite variable; the present study sought to determine if prior morphine history might account for individual differences in sensitivity to ibogaine. Female Sprague-Dawley rats, pretreated for 10 days with morphine (30 mg/kg, i.p.) before receiving ibogaine (40 mg/kg, i.p.), showed significantly less locomotor activity in response to morphine (2-5x30 mg/kg, i.p.) than controls pretreated with morphine prior to ibogaine. Thus, prior morphine exposure enhanced ibogaine antagonism of morphine-induced locomotor activity. To study whether ibogaine’s effects may be related to kappa receptor, for which ibogaine has affinity, two kappa agonists were administered using the same treatment parameters. The selective kappa agonists, U50488 (10 mg/kg, i.p.) and U69593 (1 mg/kg, i.p.), were injected in place of ibogaine, mimicked ibogaine’s effects. Furthermore, in preliminary studies, the selective kappa antagonist nor-binaltorphimine (10 mg/kg, s.c.) appeared to reverse the ibogaine-induced locomotor suppression. These data suggest that the long-lasting effects of ibogaine may be due to persisting levels of ibogaine and/or an ibogaine metabolite interacting at kappa opioid receptors to yield anti-addictive effects. (Supported by DA0817)

05.6


The mesolimbic dopaminergic system, specifically the ventral tegmental area (VTA) and its projection to target neurons in the nucleus accumbens (NAC), mediates some of the reinforcing effects of drugs of abuse. Previous results from rats forcibly treated with chronic morphine found several biochemical adaptations in the VTA and NAC (see Neuron 11995, 1993). This experiment validates the previous findings by using the animal model of drug self-administration (SA), which better approximates human drug addiction. In these studies, rats self-administered heroin in limited, daily 6 hr testing sessions for three weeks. A yoked-heroin and a yoked-saline control group were used for comparison. The findings show that opiate SA produces similar biochemical adaptations, when compared to animals receiving SA saline. These include an increase in tyrosine hydroxylase (TH) and glial fibrillary acidic protein, and a trend toward a decrease in neuropeptide Y, in the NAC. Interestingly, heroin SA produces an important adaptation in the NAC, a decrease in TH levels, not seen in animals treated chronically with morphine. There were no differences between heroin SA rats and saline SA rats.

Although the heroin self-administering rats failed to show signs of physical dependence, biochemical adaptations in the mesolimbic dopaminergic system of these rats suggest that these changes could contribute to the motivational aspects of drug addiction.
505.7 REINSTATEMENT OF HEROIN SELF-ADMINISTRATION BEHAVIOR BY EXPOSURE TO PREDILECTIONS AFTER PREFERENCE EXTINCTION

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We have shown previously that non-contingent IV priming injections of heroin, or intraperitoneal injections of morphine injected into VTA reinitiates heroin self-administration behavior in rats following a period of extinction. Naltrexone (N) given alone or given before baseline responding. Here we report that first-time exposure to 10 min of intermittent footshock reinitiates previously extinguished heroin self-administration behavior.

Male and female rats were trained to self-administer heroin (0.125-50 µg/kg/injection, IV) over four 3-h sessions per week for 2 week period. The drug-reinforced behavior was extinguished by at least 16 sessions (lever presses resulted in saline infusions). Reinstatement was studied following non-contingent infusions of saline, 50 µg/kg heroin, naltrexone-precipitated opioid withdrawal (5 mg/kg naltrexone, SC) and by 10-min of footshock (1 mA; 0.5 sec on, mean period of 40 sec).

Reinstatement was observed following footshock stress and priming injections of heroin, but not saline or naltrexone-precipitated withdrawal. Most importantly, footshock stress reinstated self-administration behavior upon first exposure, and again more than one month after the termination of heroin self-administration. These findings suggest that exposure to some stressors can precipitate relapse, possibly by mimicking the neurochemical effects of heroin self-injection and not by inducing opioid-like withdrawal symptoms.

505.8 OVARIAN HORMONES DO NOT ALTER SELF-ADMINISTRATION OF HEROIN IN OVARIETOMIZED FEMALE RATS

J. Stewart, B. C. Woodside and Y. Shaham. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

We studied the effect of ovarian hormones on the initiation and maintenance of heroin self-administration in ovariectomized female rats.

In Experiment 1, the effects of 10 µg estradiol benzoate (EB) and 0.5 mg progesterone (P) on the administration of heroin were studied. Animals were trained to self-administer heroin (50 µg/kg per injection, IV) on a progressive ratio schedule, 4 sessions per day, 4-h each. Animals were tested with descending doses of heroin (50, 25, 12.5, 6.25 µg) in 5-day blocks. Neither EB, given on the third, nor P, given on the fifth day of each block, affected the number of injections.

In Experiment 2, we studied the initiation of IV self-administration of heroin on an FR1 schedule using an ascending series of doses (0.125-50 µg/kg per injection). Following ovariectomy, 6 of 16 animals were injected with 10 µg EB on 3 occasions every 3 days before self-administration training and every 3 days throughout training. Twelve 3-h sessions per dose, 4 session per day, were given. No differences in rates of responding between groups were observed at any of the heroin doses; most animals achieved stable responding at the 25 µg/kg dose. Finally, no group differences were found when animals were tested again using a descending series of doses.

These results suggest that circulating ovarian hormones do not affect the sensitivity of female rats to the reinforcing effects of heroin.

505.9 THE DEVELOPMENT OF A CONDITIONED PLACE PREFERENCE (CPP) TO MORPHINE. 1. EFFECTS OF LESIONS OF VARIOUS CNS SITES. M.C. Olmstead and K.B. Franklin. Department of Psychology, McGill University, Montreal, Quebec, Canada.

The CPP paradigm is used to assess the reinforcing effects of incentive stimuli in that animals spend more time in the presence of cues previously associated with rewarding stimuli than those associated with neutral stimuli. In order to determine the neural substrates mediating the reinforcing effects of morphine, separate groups of male Long-Evans rats received neurotoxic lesions of various CNS sites and were tested for the development of a CPP to morphine (2 mg/kg X 3 pairings). Lesions of the hippocampus, periaqueductal gray, or pendumulopontine nucleus disrupted the CPP. Lesions of the entire ventral striatum reduced the CPP whereas selective lesions of anterior or medial portions were ineffective. Lesions of the dorsal striatum, lateral amygdala, or mesolimbic dopamine system changed the pattern of CPP behaviour but did not reduce the CPP. It appears that the development of a CPP to morphine is a complex phenomenon which depends on interactions between a number of brain structures.


Ibogaine has been proposed to serve as an agent to reduce craving for addictive agents. A series of experiments were conducted to determine the effects of ibogaine (40 mg/kg, ip) on place preference conditioning produced by morphine (5 mg/kg, ip) and on place aversion conditioning produced by naloxone (1-2 mg/kg, ip). Ibogaine alone was found to be ineffective in producing either a place preference or a place aversion; however, it did interfere with the effects of ibogaine on naloxone-induced place aversion conditioning. In addition, the ability of ibogaine to attenuate the expression of a previously learned morphine-induced place preference was assessed.

505.10 INTRAVENOUS MORPHINE SELF-ADMINISTRATION BY RATS WITH LOW VS. HIGH SACCHARIN PREFERENCES

B.A. Gouzoules, D.D. Krah, K.E. Lane and S.M. Bell. Department of Psychiatry, University of Wisconsin-Madison, Madison, WI 53792.

An experiment was performed to determine the relationship between saccharin preference and morphine self-administration. Animals received voluntary intake of a 0.1% saccharin solution in 1 hr daily sessions, low and high preference groups (n=8/group) of male Sprague-Dawley rats were selected from a larger group. Male rats were conditioned (0.5 mg/ml) was then measured in the selected rats in 1 hr daily sessions. The groups did not differ in either water-deprived or non-deprived conditions. Rats catterers were then implanted in all rats. One 6 hr session daily for 40 days was performed on 20 animals. Experience was measured in the operant chambers, during which rats were allowed to self-administer amphetamine sulphate (1 mg/kg) at unit doses beginning at 0.04 mg/kg/infusion, over the course of the experiment, the dose was increased to 0.08 mg/kg/infusion. Fourteen rats completed the study through the end of the sessions at 0.08 mg/kg/infusion, 10 completed the entire study. The groups did not differ in the number of infusions obtained at 0.04 mg/kg/infusion. Over the course of the 0.08 mg/kg sessions, saccharin-prefering rats began to self-administer significantly more morphine than rats with a low saccharin preference. For example, averaged over sessions 1-20 at this dose, the high saccharin rats obtained 10.5 ± 2.3 infusions per session, whereas the low saccharin rats obtained 4 ± 0.8 (p<0.05). Increasing the unit dose from 0.08 to 0.16 mg/kg/infusion increased an increase in self-administration in the low saccharin group and a decrease in self-administration in the high saccharin group. The positive relationship between saccharin intake and morphine self-administration may be due to the mediation by a common reward mechanism. Supported by NIDA DA05471 and DA00827.


Context-specific locomotor stimulation occurs when a distinct environment (context) previously paired with morphine elicits an increase in activity relative to a context that was explicitly unpaired. Given that this work has typically used a context conditioned stimulus (CS), it is unclear whether a context CS is necessary for locomotor stimulation, or whether other CS types would work. A context can be viewed as a polymodal stimulus composed of many elements (eg, auditory, olfactory, and tactile). The present experiment examined the ability of these context cues (odor and taste) to serve as CSs in a conditioned sensitization paradigm. Adult male Sprague-Dawley rats were used in both the taste and the odor studies. Rats received 8 conditioning trials in which a morphine baseline (4 mg/kg, SC) was immediately followed by 15-min exposure to the CS (eg, saccharin taste or banana odor) in a chamber. Using a CS-alone test to assess locomotor sensitization, we found greater activity in morphine-paired rats than in unpaired control rats (see Fig below). Thus, olfactory and gustatory elements of a context are capable of controlling Pavlovian conditioned increases in locomotor activity.

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The development of a conditioned place preference to morphine. II. Effects of microinjections into various CNS sites. K.B.L. Franklin* and M.C. Olmstead. Dept. Psychology, McGill University, Montreal, Quebec, Canada.

In the CPP paradigm, animals will approach and maintain contact with an environment previously paired with morphine administration. The present study investigated the neural sites where morphine may produce its reinforcing effect. Bilateral infusions of morphine (1 μg in 0.5 μl over 1 min X 2 pairings) into the lateral ventricles, periaqueductal gray (PAG), or ventral tegmental area (VTA) produced a CPP. Injections into the dorsal striatum, frontal cortex, hippocampus, lateral amygdala, lateral hypothalamus, pedunculopontine nucleus, posterior hypothalamus, ventral pallidum, or ventral striatum did not produce a CPP. Activation of either PAG or VTA opioid receptors is sufficient to produce morphine reinforcement but the effect may involve an interaction between the two sites.
503.3 EFFECT OF NITRIC OXIDE SYNTHASE INHIBITION ON MORPHINE-INDUCED PHYSIOLOGICAL ANXIETY AND ABSTINENCE SYNDROME IN THE RAT. George A. Matsuyuki, N.P. Plutnickoff and Hemendra N. Bhardwaj, Dept. Pharmacology and Pharmacognosy, University of Illinois, Chicago, IL 60612.

The effects of L-NAME-monomethyl-arginine (NMA) on the development of tolerance to and dependence on morphine as well as on the naloxone-induced abstinence symptoms were determined. Male Sprague-Dawley rats were made tolerant to and dependent on morphine by s.c. implantation of 4 morphine pellets during a 3-day period with 2 pellets on am of day 1 and 2 pellets on pm of day 2. NMA (2, 4 or 8 mg/kg, s.c.) was administered twice a day for 3 days. The pellets were removed on day 4 and tolerance and dependence were assessed 6 hr after the pellet removal. The development of naloxone withdrawal signs was used to evaluate the ability of both the antagonist and morphine to inhibit by 4 and 8 mg/kg doses of NMA. Physical dependence development was also inhibited as evidenced by the decrease in jumping behavior induced by naloxone (5 mg/kg, i.p.) but the other symptoms were not affected. The doses of NMA which inhibited the tolerance and dependence development were unable to inhibit the naloxone-induced withdrawal symptoms in morphine-dependent rats. It is possible that much higher doses of NMA are required to inhibit antagonist-induced withdrawal in morphine-dependent rats (Supported by a Research Scientist Development Award K02-DA-00103 from the National Institute on Drug Abuse).

503.5 ETHANOLOGY OF THE OPIATE WITHDRAWAL SYNDROME IN RATS. F.P. Espay, L. Stumy and M. Calude (1). Ence. Ciencias de la Salud, Univ. de Sevilla, Spain, (2) INSERM U.259, Univ. de Bordeaux II, Bordeaux, France.

Physical signs during the rat's opiate withdrawal syndrome are usually evaluated by the Geller-Holtzmann score. J. Pharmacol. Exp. Ther. 205: 536-546, 1978). However, this score was not elaborated according to a modern behavioral criterion, and it appears to be somewhat arbitrary. The objectives of this study were thus: i) to analyze the overall rat's behavior during the opiate withdrawal syndrome, and ii) to evaluate the validity of the calendar score. Rats were implanted with a s.c. morphine pellets (75 mg x 2, s.c.) and assigned to six groups (n = 10), receiving naloxone (0.0, 0.01, 0.05, 0.1, 0.5, 1 mg/kg, i.p.) to precipitate the withdrawal syndrome. Behavior was videotaped and analyzed by an ethological technique. Score values as well as frequency, duration and latency of each pattern were quantified. A cluster analysis allowed to discern the behavior structure. Ethogram was composed of: sniff, walk, sniffing, licking, posture, wet dog shake, seifing, grooming, gustatory, grooming, abnormal posture, swallowing, intermittent walking, jumping, teeth-chattering, freezing, elongation, yawning, and licking. Number of ejaculations, defecations and micturitions, as well as other physical signs (diarrhoea, irritability, weight loss, etc.) were also evaluated. Results revealed that abnormal posture and intermittent walking, core patterns, changed in a dose-related fashion. Wet dog shakes and jumping, main behavioral signs included in the classic score, were only enhanced in a dose-related manner with intermediate doses. Significant changes in weight loss and irritability were found to be dose-dependent. Score values were gradually enhanced after naloxone injection, except for the highest dose (1 mg/kg). In conclusion: i) the Geller-Holtzmann score is useful, but it appears not to be very accurate for high naloxone doses, and ii) abnormal reactions, weight loss and irritability are the best indicators of the opiate withdrawal syndrome level, and they might be the basis of a simpler ethological score.


To clarify the involvement of different opioid receptor subtypes in regulation of brain glutamate (Glup)/aspartate (Asp) levels during opioid withdrawal, extracellular fluid (ECF) levels of Glup/Asp were examined with in vivo microdialysis of the locus coeruleus (LC) following precipitation of withdrawal using antagonists selective for mu (CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-His-d-Phe)3 (Nor-BNI), norepinephrine (NPFF-antagonist), and levorphanol dependent SII rats, respectively. Dependance was induced by i.c.v. (i.c.v.) infusion of morphine (26 nmol/1 µl/hr), butorphanol (26 nmol/1 µl/hr), or saline (1 µl/hr) for 3 days. Microdialysis probes (2 mm tips) were inserted into the LC 24 hr before precipitation of withdrawal by i.c.v. injection of 48 nmol/5 µl of CTOP, Nor-BNI, or NTI. Behavioral evidence of withdrawal was detected following CTOP challenge in morphine and butorphanol infusd rats, and following Nor-BNI challenge in butorphanol infused rats, but not in other groups. Levels of Glup in the LC increased to 490% and 258%, respectively, above basal values in the first 15 min following CTOP injection in morphine (n=5) and butorphanol dependent (n=5) rats, while Asp was increased to 258% (n=5) and 185% (n=5) of basal levels in the same groups. Withdrawal precipitated by Nor-BNI produced significant increases in LC levels of Glup (200% of basal; n=6) and Asp (132% of basal; n=6) only in butorphanol dependent rats. No changes in EAA levels were noted following NTI injection, in any group. Opid withdrawal is associated with increased levels of EAAs, within the LC. These increases are mediated primarily through μ opioid receptors in morphine dependent rats. In contrast, both κ and µ receptor subtypes regulate EAA levels during withdrawal from butorphanol. (Supported by DA 05828).

503.7 SUBCUTANEOUS INJECTION OF AN ANALOG OF NEUROPEPTIDE FF PREVENTS NALOXONE-PREVAPRITITED MORPHINE ABSTINENCE SYNDROME. J.L. Lake, D.L. Swaim, J.A. Jones, A.F. Clauser, P.A. Suggs, K.K. Han, T. Liu, K. Burgess and D.H. Malik, Univ. of Houston-Clear Lake, Houston, TX 77058, and Texas AM University, Dept. of Chemistry, College Station, TX 77843.

Neuropeptide FF (NPF) has antipsychotic activity and play a role in opiate dependence and abstinence syndrome. Recently, the C-terminal tetrapeptide of NPF was identified as decreased in lipophilicity and penetration of the blood-brain barrier. This compound, dapsyl-[D-Phe-Arg-Met]amide, precipitated morphine abstinence following s.c. administration. In the present study, the C-terminal -Phel was removed from dapsyl-PDFAamide in an effort to convert it to a NPF-antagonist. In Exp. 1, 5 rats pre-treated with dapsyl-PDFAamide were injected subcutaneously with either 1 µg dapsyl-PDFAamide (n=6) or 20% ETUH of vehicle alone (n=6) 6 mins prior to receiving 1 mg NPF. Rats pre-treated with dapsyl-PDFAamide had a significant, p<.001, 70% decrease in morphine abstinence-like signs compared with rats pretreated with vehicle alone. In Exp. 2, 12 rats were injected subcutaneously with 13 mg/kg dapsyl-PDFAamide (n=5) or 20% ETUH of vehicle alone (n=5) 5 mins prior to receiving 0.125 mg/kg naloxone. Rats injected with dapsyl-PDFAamide exhibited a significant, p<.001, 70% reduction in overall abstinence signs as compared with rats receiving vehicle alone. (NIDA DA06553 and Texas Advanced Technology Program.)

503.8 INHIBITION OF MORPHINE TOLERANCE AND DEPENDENCE BY DIAZEPAM AND ITS RELATION TO CYCLIC AMP LEVELS IN THE RAT BRAIN. M.-L. Shao, P. Sribandilmongkol, D. Santos and G.A. Tejovs, Dept. of Pharmacology, College of Medicine, Ohio State University, Columbus, OH 43210.

We have recently observed that met-enkephalin may be involved in the inhibition of morphine tolerance and dependence by diazepam (Sribandilmongkol et al. 1993). In previous studies, cyclic AMP may also play an important role in this phenomenon. Male Sprague-Dawley rats were made tolerant-dependent by s.c. implantation of six morphine pellets. Diazepam (0.25 mg/kg body wt.) was injected i.p. in some rats. Control rats were implanted with placebo pellets and injected daily with saline or diazepam. Morphine tolerant rats were sacrificed 1 h. after injections. Abstinence was induced by injecting s.c. naloxone (10 mg/kg) and withdrawal effects were observed for 30 min. There was no change in cyclic AMP levels in hypothalamus, striatum or cortex of morphine-tolerant animals compared to rats given placebo. Diazepam had no effect on cyclic AMP levels in these animals. Morphine abstinence rats showed a significant increase in cyclic AMP levels in striatum (71%), cortex (126%) compared to placebo treated rats. This increase in cyclic AMP levels preceded the morphine abstinence abstinence rats injected with diazepam. It is concluded that cyclic AMP may be involved in the inhibition of morphine dependence by diazepam.
503.11

ABSTINENCE PRECIPITATED BY NALOXONE FOLLOWING A SINGLE DOSE OF MORPHINE: POTENTIATION FOLLOWING A SECOND EXPOSURE. G.J. Herbert, G.F. Koch, and O. Schultes, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Recent studies in humans with no prior history of opiate abuse reported naloxone-precipitated withdrawal signs following a single exposure to morphine, and this effect was markedly enhanced following a second morphine exposure (Avrontza et al., Psychopharmacology, 114:71, 1994). In a recent report using an established animal model of this acute dependence-like phenomenon, the response disruptive effects of naloxone on opiate withdrawal were enhanced 4 hrs after a single injection of morphine, with a further potentiation of this effect following weekly exposures to morphine and naloxone for 2 months (Adams & Holtzman, J Pharmacol Exp Ther, 253:483, 1990). Using a similar operant procedure, the present study sought to determine if this increase in sensitivity to an opiate agonist could be detected after a single exposure only to morphine exposures, as seen with humans. Rats were trained to lever press for food on an FR-15 schedule of reinforcement. Rats were pretrained with morphine (2.5, or 10 mg/kg s.c.) followed 4 hours later by an injection of naloxone (0 - 3.0 mg/kg s.c.). Prior morphine administration dose-dependently increased naloxone's response disruptive effects relative to morphine-naive baseline, and this effect was markedly potentiated following a second morphine/naloxone exposure 7 days later. The current results confirm previous reports in humans and rats that withdrawal-like signs may be elicited following acute exposure to opiates, and demonstrate a potentiation of this effect with a second exposure. These findings suggest that the development of dependence on opiates as defined by the manifestation of precipitated withdrawal is a progressive phenomenon that may begin with a single dosing. Supported by grant DA04043.

503.13

Clonidine Antagonizes The Rewarding Effects Of Morphine Only In Opiate Withdrawn Rats As Well As The Aversive Effects Of Opiate Withdrawal Itself. K. Nader* and D. van der Kooy, Neurobiology Research Group, Dept. of Anatomy, Univ. of Toronto, Toronto, Ontario, MSS IAR.

Lesions of the brainstem tegmental pedunculopontine nucleus block morphine rewarding effects in drug-naive animals. Conversely, dopamine antagonists block the rewarding effects of morphine only in opiate deprived rats, as well as the aversive effects of morphine withdrawal itself. As clonidine, an α2-adrenergic receptor agonist, has been reported to antagonize the somatic signs of opiate withdrawal, we asked if clonidine would block the motivational effects of opiate withdrawal in rats as well as the aversive effects of morphine withdrawal itself. As clonidine, an α2-adrenergic receptor agonist, has been reported to antagonize the somatic signs of opiate withdrawal, we asked if clonidine would block the motivational effects of opiate withdrawal in rats as well as the aversive effects of morphine withdrawal itself, but did not affect morphine place preferences (2 and 20 mg/kg) in previously drug naive rats. These results suggest that the motivational system activates those dopaminergic and noradrenergic components that are in series with each other. Furthermore, clonidine blocked the acquisition of morphine (15 mg/kg), but not LiCl (15 mg/kg), conditioned taste aversions. Given that dopamine antagonists also block morphine, but not LiCl, conditioned taste aversions, we suggest that the aversive effects of both opiate withdrawal and morphine conditioned taste aversions are mediated by the same neurobiological substrates.

503.10


There are few reports on the morphine withdrawal syndrome in the developing animal. In this study, we examined the behavioral effects of precipitated withdrawal in morphine dependent 7, 14, 21, and 42 day old rats. On the first treatment day group, different litters were removed from the dam (except for preweaning rats which were housed separately) and individual rats were injected with morphine sulfate (10 mg/kg, i.p., b.i.d., 7 day-old pups also received a dose of 3 mg/kg, i.p.) for 6.5 days. Controls included saline injected groups. The last injection was on the morning of the 7th day. For preweaning rats, two hours after the last morphine injection, each subject received one of several doses of naltrexone (0, 0.3, 1.0, 3.0, 10.0, mg/kg) and observed in a blind manner with the remainder of the litter for a total of 20 minutes. Ongoing behaviors were recorded every 15 seconds. When the observation period ended, the pup was anesthetized and placed back into the observation chamber with the remainder of the litter. Preweaning rats were tested individually on fresh bedding in the same manner as preweaning pups. Naltrexone injected pups that had been chronically treated with morphine displayed greatly increased specific behaviors and were activated. The results also show that morphine abstinence animals demonstrate withdrawal behaviors that are appropriate to the age group examined. For example, morphine abstinence 7 day-old pups show increased head movements, rolling, wall climbing, and paw movements. Morphine treated 42 day-old animals displayed the classic adult-like behaviors. These results clearly show that a morphine withdrawal-like syndrome can be described in the very young rat and that this syndrome is reflective of the specific behavioral repertoire appropriate to the age of the animal. (Supported in part by DA-06600).

503.12

CLOCINNAMOX STUDIES IN MORPHINE-TOLERANT AND NONTOLERANT RATS TRAINED TO DISCRIMINATE MORPHINE. E.A. Walker*, T.M. Richardson, Y. Wu and A.M. Young, Dept. of Psychology, Wayne State Univ., Detroit, MI 48202.

The effects of irreversible opioid antagonist clocinnamox (C-CAM) on morphine (MS) and fentanyl (PT) were compared in rats before and after repeated injections of MS to determine the role of agonist efficacy in tolerance. Male, Sprague-Dawley rats (n=20) were trained to discriminate 3.2 mg/kg MS from saline under a FR 15 schedule of food reinforcement. Cumulative doses of MS (0.32-10 mg/kg) or PT (0.0032-0.056 mg/kg) produced MS-appropriate responding and decreased response rates. In nontolerant rats, 10 mg/kg C-CAM decreased MS potency by 7-fold, but failed to significantly alter PT potency to produce MS-like stimulus effects. Repeated treatment with 20 mg/kg/day MS for 7-14 days decreased MS and PT potency to produce MS-like stimulus effects by approximately 4-fold. In these MS-tolerant rats, 10 mg/kg C-CAM further decreased MS and PT potency to produce MS-like stimulus effects by an additional 8-fold. No withdrawal signs were observed after C-CAM administration. Quantitative analysis of these data suggest that chronic opioid treatment may increase the apparent efficacy required for an agonist to exert MS-like discriminative stimulus effects. (Supported by DA03796 and KO2 DA00132).

503.14

SHORT-TERM TOLERANCE DEVELOPS BEFORE LONG-TERM TOLERANCE IN RATS. H. Burton and Catherine F. Cramer*, Dept. of Psychology, Dartmouth College, Hanover, NH 03756.

The development of tolerance to morphine analgesia was investigated in three different series with the experimental paradigms of "short-term" and "long-term" mechanisms. "Short-term" tolerance occurs with closely spaced injections of high doses, whereas, "long-term" tolerance is produced by low to moderate doses at widely spaced intervals (Taylor & Baker, 1985). In the first two experiments Long-Evans hooded rats received i.p. injections beginning on Day 1 and continuing through Day 7. Animals in one condition received on each day the injection, pups were tested for latency to retract a hindpaw from a 5°C hotplate. On Day 7 the animals were divided into four groups; first group received saline, second group received on the day of the test 10 mg/kg of morphine and were tested for analogue 20 min. following the injection. The saline condition was 10 mg/kg naloxone and a saline control. These groups were then tested for hyperalgesia, weight changes (1 and 4 hours postinjection) and locomotor behavior.

In Experiment 1, pups received 0.1, 0.2, 0.5, 1.0 or 10.0 mg/kg of morphine once a day on Day 1 and the results from four groups (2 followed by the effects of withdrawal itself) were analyzed. In Experiment 1, the exception that dosages were 0.6, 1.0, 3.0, 15.0 mg/kg, twice daily, 8 hours apart. In Experiment 3, the pups also received two daily injections of either 0, 0.6, 1.0, 3.0, or 10.0 mg/kg of morphine but were tested on Day 3. In Experiment 1, the results from each condition was compared to the baseline condition. Under long-term parameters tolerance was not observed at Day 7. The results of Experiment 2 show shifts in the affective indicators of tolerance. Withdrawal was not observed in either of the first two experiments. In Experiment 3, a shift in the dose-response curve was observed on Day 3 and withdrawal and baseline were indicated by both hyperalgesia and hyperactivity.

In sum, neonatal rats seem capable of short-term, but not long-term, tolerance during the first week, perhaps due to the nonassociative properties of the short-term paradigm.
MORPHINE WITHDRAWAL IMPAIRS ACQUISITION OF A MORRIS WATER MAZE TASK: POTENTIAL INVOLVEMENT OF STRIATAL MEDITORY. K. D. Olenic, T. J. Walsh, K. Crenshaw, S. Bailey. Rutgers University, New Brunswick, NJ 08901; *GUNN, Piscataway, NJ 08854. Behavioral and neurochemical changes in rats during early and longer-term morphine withdrawal were investigated. Experimental subjects (M) were implanted with osmotic pumps (8.75 mg/240 /µL/day over 7 days). Controls (C) received sham implants of similar size and weight. One or 21 days following pump removal, subjects were tested in for 8 days in a Morris water maze (MWM) task. Rats were sacrificed and high affinity choline transport was assessed in the hippocampus (HPC) and striatum (STR). M subjects exhibited a significant decrease in body weight during withdrawal that recovered by 21 days after pump removal. M subjects trained during early withdrawal exhibited significantly longer escape latencies due to a possible failure of procedural memory. However, during sequential probe trials these subjects exhibited a significant preference for the target area and accurate search strategies suggesting intact declarative memory. Nine days after removal of the osmotic pumps, NACH in M subjects was elevated in the STR but not in the HPC. M rats trained during longer-term withdrawal exhibited no deficits in any measure of NACH performance. NACH in these rats was comparable to C levels in both the HPC and STR. Early morphine withdrawal was accompanied by increased striatal NACH and impaired NAM acquisition.

TOLERANCE AND CROSS-TOLERANCE TO CANNABINOID LIQUIDS AFTER CHRONIC ADMINISTRATION OF ANANANDAMIDES. E. Frise and R. Mechoulam, The Hebrew University of Jerusalem, Israel. The development of tolerance to cannabinoid receptor ligands has been well established. However, it is now known whether repeated injections of the recently discovered endogenous cannabinoid ligands, also induce tolerance to subsequent administration of anandamides or cannabinoid drugs. Ten daily injections (p.o.) of anandamide (20, 40, 80, 160, and 400 mg/kg) were administered to adult female C5/BL mice. Twenty-four hr after the last injection, animals were challenged with 20 mg/kg of MOR on a 5TH cycle and tested for a latency of response to 5TH. These results indicate that repeated administration of anandamides induces tolerance to cannabinoid receptor ligands. Further, the tendency for increased sensitivity to 5TH after chronic administration of a very low dose of ana- 

ELEVATIONS AND PHASIC FLUCTUATIONS IN NUCLEUS ACCUMBENS DOPAMINE (DA) DURING IV HERON SELF- ADMINISTRATION. D. Poblocki, K. Lebo, and R. A. Wise. Cr Stud Behav Neurosci Montréal, PQ Canada H3G 1M8. Fluctuations in extracellular nucleus accumbens DA and DOPAC levels were measured in 4-min microdialysis samples from rats engaged in IV heroin self-administration. DA levels were elevated to 200-400% of baseline during self-administration, fluctuating phasically between responses. Each injection caused a short-latency increase in DA levels; a second increase preceded the next lever-press. Drug requests (lever-presses) normally occurred well before DA levels fell to near-normal levels. DOPAC levels increased in the first minutes and remained elevated during the period of drug availability, showing no significant fluctuations that were time-locked to the response cycle. These observations confirm that self-administered IV doses of heroin are sufficient to cause the next lever-press. Drug requests (lever-presses) normally occurred well before DA levels fell to near-normal levels. DOPAC levels increased in the first minutes and remained elevated during the period of drug availability, showing no significant fluctuations that were time-locked to the response cycle. These observations confirm that self-administered IV doses of heroin are sufficient to cause the next lever-press.
DIFFERENTIAL EFFECTS OF DOI ON COCAINE (COC) AND MORPHINE (MOR)-INDUCED EXTRACELLULAR DOPAMINE (DA) RELEASE IN THE NUCLEUS ACCUMBENS (NA) D.L. Williams*, J. Ishikawa, J.A. Garcia, J. Dai and H.L.Y. Melzer. Department of Psychiatry, Case Western Reserve University, Cleveland, Ohio 44106

There is previous evidence that blockade of 5-HT2 receptors can inhibit the effect of COC and MOR to increase extracellular DA in the NA. The purpose of this study was to determine if DOI, a direct acting 5-HT2A/2C agonist, can influence the effect of COC and MOR on extracellular DA in the NA in awake rats using microdialysis. The systemic administration of DOI (2.5 mg/kg, s.c.) alone had no effect on basal extracellular NA DA levels. COC (10 mg/kg, i.p.) produced an increase in extracellular DA in the NA of 333% over baseline. Pretreatment of rats with DOI 30 min. prior to the administration of COC did not significantly alter the increase in extracellular DA produced by COC. MOR (5mg/kg, s.c.) produced an increase in extracellular DA in the NA of 206% over baseline. DOI pretreatment 30 min. prior to MOR inhibited the MOR-induced increase in extracellular DA. These findings suggest that MOR and COC-induced increases in extracellular DA in the NA are regulated differently by the activation of 5HT2A/2C receptors. The ability of DOI to suppress MOR-induced DA release in the NA suggests that non-hallucinogenic 5-HT2 agonists may have a role in the treatment of addiction.

EVIDENCE FOR A WITHDRAWAL SYNDROME FOLLOWING CHRONIC ADMINISTRATION OF AN ANABOLIC STEROID TO RATS. Katherine R. Benson, Nancy A. Carrick* and Dennis L. Murphy. Laboratory of Clinical Science, National Institute of Mental Health, Building 10, Room 3D41, 9000 Rockville Pike, Bethesda, MD 20892.

In the past decade, anabolic steroids have been recognized as drugs of abuse, especially among athletes and body builders. Chronic administration of testosterone and other androgens has been shown to induce behavioral changes in rats and in humans which includes increases in aggression and alterations in mood. A putative withdrawal syndrome following chronic self-administration of anabolic steroids has been described in humans but has not been demonstrated experimentally in either humans or rats. The present study shows that distinct behavioral effects emerge in two strains of rats following withdrawal from long-term but not short term administration of testosterone propionate (TP) (30 mg/kg/day). In both Wistar and Fawn-Hooded rats, withdrawal from TP following ten weeks of daily administration induced a behavioral syndrome characterized by facial tremor, head- weaving, full-body lurches and dizziness. This syndrome appeared to be of a greater intensity in the Fawn-Hooded strain. These behaviors subsided within two weeks of withdrawal from the androgen. There were no significant changes in temperature, food intake or body weight over the same period. In comparison, three weeks of daily TP administration did not produce a consistent behavioral syndrome following withdrawal in either Wistar or Fawn-Hooded rats, although there was a significant increase in yawning behavior. These results show evidence for the first time of a withdrawal syndrome in rats following chronic administration of high dose anabolic steroids.

THE LOCAL CEREBRAL METABOLIC EFFECTS OF MORPHINE SENSITIZATION IN THE RAT. M.A. Kraus*, J.M. Piper, R.T. Livsey and C. Kornetsky. Lab. of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Repeated administration of morphine sulfate (MS) will sensitize a rat to oral stereotypic effects. The effects of this sensitization on local cerebral metabolic rate of glucose (LCMRglu) was determined in male F-344 rats. Rats were sensitized by four 10 mg/kg MS (sc) injections at 12 hr intervals. Two control groups were administered saline. One week later, the sensitized animals and one control group, morphine-control, were administered 0.5 mg/kg MS 10 min prior to the 2-[14C]deoxyglucose bolus. The second control group received saline in place of MS. LCMRglu in 49 brain sites was significantly greater in the sensitized rats than the morphine-control rats after the MS challenge. There also were increases in LCMRglu in sensitized animals compared to the saline-controls in a number of structures. These results suggest that MS sensitization involves increased metabolic activity in multiple brain systems including mesolimbic and motor structures. (Supported by Grants DA03236 and DA00099 to CK)

NICOTINE INCREASES MET-ENKEPHALIN CONTENT AND PREPROENKEPHALIN mRNA IN MOUSE STRIATUM. N.H. Nett*, R.K. Dhatt, T.A. Wemminger, C.A. Tejwani and M. Hadjiconstantinou. Dept.s of Pharmacology, Psychiatry and The Neurosciences Program, Ohio State University College of Medicine, Columbus, Ohio 43210.

Behavioral evidence suggests that opioids play a role in nicotine addiction. Indeed, naloxone reportedly can precipitate withdrawal behavior in animals treated with nicotine chronically. We now present evidence that acute or chronic administration of nicotine increases met-enkephalin immunoreactivity (Met-Enkli) in the mouse striatum. The response to a single dose of nicotine is time-dependent, with Met-Enk content reaching a maximum 30-60 min post-injection and returning to near normal by 6 hr. The nicotine-induced increase of Met-Enkli is blocked by pretreatment with the nicotinic antagonist mecamylamine, but not by the muscarinic antagonist atropine. Daily administration of nicotine for 2 weeks also increases striatal Met-Enkli. In addition to increasing Met-Enk content, chronic nicotine increases preproenkephalin mRNA in the striatum when assayed after 7 or 14 days of treatment. We postulate that nicotine addiction is associated with enhanced brain content of opioids which could explain withdrawal behavior when nicotine addicted animals are treated with naloxone.

THE EFFECT OF NALOXONE, MORPHINE, AND THEIR COMBINATION ON LOCAL CEREBRAL METABOLIC RATES FOR GLUCOSE IN THE RAT. C. Kornetsky*, M.A. Kraus and J.M. Piper. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

In a present experiment used 2-deoxy-D-[14C]glucose (2-DG) to determine the effects of naloxone (NX), morphine sulfate (MS), and the combination treatment of NX-MS on local cerebral metabolic rates for glucose (LCMRglu) in rats. Four groups were studied (N = 18) saline, NX, MS, or NX-MS. One day prior to the NX-MS, NX by itself, significantly decreased LCMRglu in the locus coeruleus, a structure believed to be involved in the expression of opiate withdrawal, suggesting a role of endogenous opioids in the regulation of central noradrenergic activity. Also, NX significantly increased LCMRglu in the mediodorsal thalamic nucleus which has extensive reciprocal connections with the limbic system and prefrontal cortex, suggesting an impact on the integration of limbic and cortical activity. Finally, NX blocked the inhibitory effects of MS on LCMRglu. (Supported by Grants DA03236 and DA00099 to CK).
504.3
MOLECULAR MODELING OF ANANDAMIDE V.M.
Showalter and M.E. Aboud* Dept. of 
Pharmacology and Toxicology, Virginia Commonwealth
University, Richmond, VA 23298.

Other investigators have utilized molecular modeling to 
gain more insight into how cannabinoid ligands bind to 
their receptor. Anandamide (arachidonylethanolamine) is thought 
to be the endogenous ligand at cannabinoid receptors. The 
purpose of this study was to extend the cannabinoid 
pharmacophore to include anandamide. The structures of 
cannabinoid ligands were modeled using SYBYL. The 
structure of arachidonylethanolamine (anandamide, AEA) was 
constructed based on x-ray crystal data for 9-THC. 
The structure of anandamide was generated by adding 
ethanolamine to the cannabinoid acid, 

A9-THC. A linear 
systematic search was performed to find the 


504.5
CHARACTERIZATION OF A CANNABINOID RECEPTOR IN 
THE ROUGH-SKIN NEWT, K. Soderton*, F.L. Moore*, P.H. Franklin* 
and T.V. Murray. College of Pharmacy, and Department of Zoology, 
Oregon State University, Corvallis OR 97331

Binding of the synthetic cannabinoid [3H]CP-55940 to membranes 
prepared from brains of rough-skinned newts (Taricha granulosa) 
was investigated using a rapid filtration 
assay. Binding equilibrium was reached 
by 480 min at 15°C. Specific binding was saturable whereas non-specific 
binding (determined in presence of 10 μM levonantradol) was linearly 
related to [3H]CP-55940 concentration. Saturation 

isotherms provided a Kd value of 10.5 nM and Bmax of 1.64 pmol/mg protein. 

Estimates of Ki obtained by kinetic analyses agreed with equilibrium 
saturation results. Competition experiments using unlabeled 
CP-55940 and levonantradol 
yielded Ki values of 8.6 nM and 5.9 nM respectively. Affinity differences 
between the amphibian receptor and values previously reported for the rat 
(0.2-4.0 nM) may be explained by differences in incubation temperatures; 
15°C for the newt vs. 30°C for the rat. Using a rat brain membrane 
preparation we found that Ki varied inversely with temperature; 

Kd = 12.5, 6.5, and 3.9 nM at 15, 15, and 30°C. Behavioral experiments suggest that 
the cannabinoid binding site has physiological relevance. News treated 
with 5 μg of levonantradol IP displayed significantly decreased locomotor 
activity (measured in crossings of lines painted on a testing-arena floor 
during a three minute period). Treated animals averaged 4.1 line crossings vs. 11.4 in 
vehicle controls (p < 0.01, 2-tailed t-test). These data 
suggest that rough-skinned newt is a useful system for the 
study of cannabinoid pharmacology.

504.6
ANANDAMIDE AND Δ9-THC DILATION OF IN VIVO CEREBRAL 
ARTERIOLES IS BLOCKED BY INDOMETHACIN. S. F. Moore, K.A. 
Withgottby and E.F. Ellis Department of Pharmacology and Toxicology, 
Medical College of Virginia, Richmond, VA 23284-2913

An endogenous ligand of the Δ9-THC receptor, arachidonylethanolamine (anandamide, AN) has been shown to cause some behavioral 
and physiological alterations similar to those induced by Δ9-THC. We 
investigated the effect of increasing, topically applied doses (10-12, 10-13 M) 
of AN and Δ9-THC on in vivo cerebral arterial diameter using the acute cranial 
window technique in anesthetized rabbits. Both AN and Δ9-THC induce a dose-dependent dilation, with Δ9-THC being more 
active at doses as low as 10-11 M. Dilation by both agents was completely 
blocked by topical application of the cyclooxygenase inhibitor 
indomethacin. Superoxide dismutase plus catalase, which block cyclooxygenase-dependent dilation produced by bradykinin or arachidonic acid, 
were inactive on AN- and Δ9-THC-induced dilation, implying dilation 
is not via vasoactive, dilator oxygen radicals. HPLC analysis of Δ9-THAN passed 
under the cranial window showed approximately 20% metabolism to Δ9-
arachidonic acid (AA). Following IV injection of Δ9-THAN in mice both Δ9-THAN 
and Δ9-AA were found in brain. Reported increases in cerebral blood flow 
caused by marijuana may be related to formation of brain eicosanoids. 
Endogenous AN may modulate cerebral blood flow.

Supported by DA 07007 and a Javits Neuroscience Investigator Award.

504.7
BINDING OF [3H]MK-801 TO BRAIN REGIONS AND SPINAL 
CORD OF MICE AND RATS TREATED CHRONICALLY WITH 
MORPHINE. Krishnamurthy P. Gadhadhith*, Poluru L. Reddy 
and Hemendra N. Bhargava, Dept. Pharmacometrics and 
Pharmacodynamics, Univ. Ill at Chicago, Chicago, Ill. 60612

The effect of morphine tolerance-dependence and abstinence on the 
binding of [3H]MK-801 to brain regions and spinal cord of mice and 
rats was determined. Male Swiss-Webster mice and Sprague-Dawley 
rats were rendered tolerant-dependent by implanting a pellet (mice) 
containing 75 mg of morphine base for 3 days and 6 pellets during a 
7-day period (rats), respectively. In tolerant-dependent animals, the 

pellets were left intact where in abstinence animals pellets were removed 
6 hr (mice) and 16 hr (rats), respectively, before sacrificing. In the absence of 
glucocorticoids, the binding (Rm) of [3H]MK-801 was 
decreased by 10% in the cerebral cortex of morphine tolerant but not 
in the abstinence rats. In the presence of glucocorticoids, the binding 
was decreased in midbrain and spinal cord of tolerant rats, 
and in hypothalamus, midbrain and spinal cord of morphine 
atabstinent rats. In morphine tolerant mice, the binding of [3H]MK-801 was 
decreased in pons-medulla and hypothalamus but was increased in spinal 
cord. In morphine abstinent mice, the binding of [3H]MK-801 was 
increased in hippocampus. It is concluded that chronic treatment of 
mice and rats with morphine produces differential effects on central 
NMDA receptors. Additionally, different changes are seen when the 

binding is done in the absence or presence of glucocorticoids and glutamate 
(Supported by a Research Scientist Development Award K02-DA-00130 
from the National Institute on Drug Abuse).

504.8
PRENATAL EXPOSURE TO MORPHINE ALTERS BRAIN μ OPIOD 
RECEPTOR CHARACTERISTICS IN ADULT FEMALE RATS. 
Agnes Rimaniclay* and Ilona Vathy*. Dept. Psychiatry, Albert Einstein 
College of Medicine, Bronx, NY 10461. Dept. Psychiatry, A. Sz-Gyorgyi Medical 
School, Szeged, Hungary.

The present study investigated the opioid binding characteristics of 
membranes from different brain regions of adult female rats exposed to morphine 
in utero (10 mg/kgpricwice daily on days 11-18 of gestation). Females 
were overanesthetized (OVX) at least one week prior to sacrifice, and some females 
were injected with 3 μg of estradiol benzoate (EB) 48 hours before sacrifice. 
Saturating binding assays were carried out using the N labeled, highly selective 
ligand D-Ala-Gly-N-Methyl-Phe-Gly-ol (DAMGO) to label μ opioid receptors. 
The maximal binding capacity (Bm) and affinity (Kd) were estimated in the 
hypothalamus (HYP), preoptic area (POA), frontal cortex (CX), striatum 
(STR), ventral parietal area (YTA) and cerebellum (CER). Prenatal exposure 
to morphine reduced the Bm and the Kd about 25% in the HYP of OVX 
females when compared to OVX, saline-treated females. A 3 μg EB injection 
increased the Bm and the Kd about 25% in the HYP of morphine-exposed 
females without affecting saline-treated females. These effects of prenatal 
morphine were not apparent in either the Bm or the Kd in the POA, CX, STR, 
YTA or CER. The increase in hypothalamic μ receptor binding capacity 
in prenatally morphine-exposed females in response to estrogen treatment may 
be related to the deficit seen in adult sexual behavior of prenatally morphine-
exposed females in a previous study (Vathy and Katay, Dev. Brain Res., 
68:125-131, 1992). Supported by DA 05833 awarded to IV.
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WEDNESDAY PM
DRUGS OF ABUSE: METABOLISM—PHARMACOLOGY

1237

S05.4


Toluene (T) is inhauful for its euphoric effects. We reported that T markedly enhanced evoked responses elicited by single pulses in the hippocampal slice preparation (Neurosci. Abstr. 19(2), 1,461, 1993). This effect was antagonized by GABA and muscimol. To explore further the interactionsee pharmacology, we also examined the effects of T on responses elicited by paired-pulses and its interaction with compounds affecting the GABA and GLU neurotransmitter systems. Responses were elicited by paired-pulse stimulation (0.1 Hz) of Schaffer collaterals in the stratum radiatum were recorded from pyramidal cells of CA1 area in transverse hippocampal slices from mice. Paired stimuli, separated by an interstimulus interval (ISI), were delivered temporally interopposite with the single-pulse train. In this paradigm, there was an increase in the response to the second relative to the first pulse (facilitation). T enhanced paired-pulse facilitation. This enhancement was antagonized by GABA and muscimol, but not by baclofen. The competitive NMDA antagonist CGS-17955 was without effect on T-enhanced facilitation; however, T was markedly enhanced paired-pulse facilitation, suggesting a residual effect of T. CNQX, an antagonist at the quisqualate site, blocked the response to single- and paired-pulses, alone and with T. After washout of CNQX and T, the response to single pulses partially recovered, whereas paired-pulse facilitation was markedly enhanced. These results further characterize the modulatory influences of other systems in the brain, possibly related to its psychoactive effects.

DEVELOPMENTAL: CORTICAL INJURY MODELS

S05.1


We have been using the rabbit with chronic kaolin-induced hydrocephalus to assess the factors determining ventricular septum opertational shunting for infantile hydrocephalus. To this end we have been monitoring the levels of behavioral, biological, cervical and anatomical toxicants in the serum of the operated and non-operated animals. In the current study the effects of hydrocephalus on the levels of tyrosine, tryptophan and SHAA were determined by HPLC with Fluorometric detection in 6 day old normal (N=14) and in 26 to 69 day old hydrocephalic (N=11) rabbits. Samples of cerebrospinal fluid (50 & 100 µl) were obtained after percutaneous injection in the cisterna magna or directly from the lateral ventricle at the time of sham insertion. Samples were stored at -80°C until processed. The hydrocephalic rabbits showed significant decreases in both tyrosine and tryptophan levels and a highly significant increase in the serotonin metabolite SHAA. There was a significant positive correlation between the intracranial pressure (ICP) and the increase in SHAA, but not with the other two amino acids. These data suggest that the increased ICP adversely affects the mechanism of removal of the serotonin metabolite from the cerebrospinal fluid.

S05.2


Rabbits with chronic kaolin-induced hydrocephalus have few neurological deficits despite profound ventriculomegaly. We report here both structural and metabolic sparing in the somatosensory cortex in chronic untreated animals. Hydrocephalus was induced at 4-6 days of age. Control (N=15) untreated hydrocephalic animals were sacrificed at 4-6 days of age. Treatment (N=15) rats were sacrificed at 40, 60 and 90 days. Upon sacrifice, brains were processed by coronal sections and/or analysis of the whole brain. The brains were processed for quantitative 2-deoxy-D-glucose autoradiography and cytochrome oxidase (N=193), stained with cresyl violet for somatosensory cortex barrel fields (N=14), or silver impregnated for quantitative neuronal golgi (N=8).

High resolution morphometric and oxidative metabolism were significantly higher in the chronic hydrocephalic group than normal, with the highest metabolic activity in the outer cortical layers in both groups, while the lowest activity was seen in the white matter. The organization of the barrel fields in the facial cortex of the chronic hydrocephalic group was preserved. The areas of the individual fields were significantly larger in the hydrocephalic than in the normal littermates. Shunted animals showed intermediate sized fields. In the hydrocephalic the apical dendrites of the layer IV neurons remained perpendicular to the cortical surface, but the dendritic shafts were thickened and the spines much more prominent. These data show that with sufficient ventriculomegaly, the structural organization of the cortex is maintained, and there is considerable functional activity in the cortical mantle of the chronic hydrocephalic rabbit.

S05.3


Hydrocephalus in childhood is marked clinical improvement but little is known about the cellular effects of hydrocephalus and the response to shunting. In this study we examined the effects of hydrocephalus and treatment with formation in postnatal and early stages of development. In this study we examined the effects of hydrocephalus and treatment in pediatric hydrocephalus rats (p=7) at 21 days of age and from age-matched control (n=6) and hydrocephalic (p=6) rats at 21 days. The brains were fixed in 10% formalin and embedded in paraffin. Sections were stained by cresyl violet and Nissl and were evaluated histologically. The results showed that hydrocephalic and shunted rats had similar tissue morphology and cytoarchitecture compared to control. However, there were differences in the distribution of neurons in the cortex and in the subcortical structures. The results indicate that the effects of hydrocephalus on the brain are not as severe as previously thought and that the effects of treatment are beneficial.

S05.4

HIGH RESOLUTION 1H-NMR ANALYSIS OF CEREBRAL CORTEX FROM CONTROL AND HYDROCEPHALIC H-TX RATS. N. G. HARRIS, R. A. INGOLD, R. C. JONES, R. W. BROWN. Dept. of Pharmacology, Biochemistry and Radiology, University of Florida, Gainesville, FL 32610 USA.

The H-Tx rat develops hydrocephalus in late gestation due to an obstruction of the cerebral aqueduct and if left untreated, affected animals die within 4-6 weeks after birth. By 21 days there is gross lateral ventricular expansion and severe cortical thinning with abnormal cell morphology. Cerebral glucose metabolism and blood flow (CBF) are greatly reduced so that in some areas CBF is below the ischemic threshold. Although many of these gross pathophysiological changes are reversible by the placement of a ventriculocisternostomy shunt soon after birth, it is not known to what extent the shunted brain is able to function normally. In an attempt to provide baseline information on the more discrete, biochemical changes that occur in the untreated hydrocephalic rat cortex, we have used high resolution 1-dimensional 1H-NMR spectroscopy to analyze cerebral extracts of cortex prepared by the freeze-focused technique using spontaneously-breathing control and hydrocephalic rats at 21 days after birth (n=4-8). Many cerebral metabolite concentrations in the hydrocephalic cortex were significantly reduced from control. N-acetyl aspartate (NAA) at 2.02ppm was 1.50±0.04µmol/g wet weight (means±SEM) in the hydrocephalic cortex compared to 3.90±0.17 in the control (P<0.01), creatine/phosphocreatine at 3.06ppm was 2.60±0.13 and 19.30±3.2 (P<0.01) in hydrocephalic and control cortex respectively and lactate at 3.44ppm was 3.60±0.51 and 7.60±0.79 (P<0.01). The following metabolites were also significantly reduced: alanine, glutamate, aspartate, glycerophosphocholine/ phosphocholine, inositol. These results confirm our previous measurements of a disrupted cortical metabolism and further indicate that the biochemistry of the hydrocephalic cortex is severely disturbed. This data establishes a good baseline from which subtle differences in the biochemistry of the shunted hydrocephalic cortex can be investigated.
CONJUNCTIVE EYE MOVEMENTS IN CEREBRAL PALSY.
M.E. Garek, S. Lee, and H. Zhang. Dept of Psychology and Biomedical Engineering Program, California State University, Sacramento, CA 95819.

Do the conjunctive movements of the eyes in cerebral palsy (CP) differ from the normal? If so, this deficit would affect visual sense in CP. The right and left eye positions of 6 CP and 6 normal (N) adults were measured using the videoelectrooculogram (VEOG) (Micromeasurements, Inc., Farmington CT). Square, sine and triangle waveforms in the time domain were utilized for 24, 10sec tests of horizontal and vertical saccadic and pursuit movements (0.5, 0.5 Hz; +4, -8 degrees). The pooled mean coefficient of determination (r²) for right and left eye positions across all tests for N ranged from 0.96±0.03 to 0.96±0.006 and for CP from 0.80±0.24 to 0.90±0.08. The mean r² was significantly lower for CP than N on all tests (Mann-Whitney U=p<0.02) except for horizontal saccades. The degree of binocularity across CP subjects did not coincide with the severity of the disorder. While the final common path for coupling the two eyes' positions may be in the tegmentum and pons, these results indicate that this visuomotor feature is not necessarily conserved in CP and its contribution to saccadic and pursuit movements may be critical.

(Supported by NCSB910726 and a CSU-S/CA Award to ML.)
DEVELOPMENTAL DISORDERS: CORTICAL INJURY MODELS


Focal development neocortical anomalies, including molecular layer ectopies and microgyria, are associated with developmental outcome. In an attempt to further test the dissociation of the behavioral effects of autoimmunity and of cortical malformation we induced ectopies (with punctate wounds) and microgyria (with freezing lesions) in the neocortex of one-day-old mice without immune disorders. DBA mice received either a puncture wound or freezing lesion of either the left or right hemisphere. Some mice received sham surgery. Compared to sham-operated animals, mice with either puncture wounds or freezing lesions performed poorly in discrimination learning, in a spatial Match-to-Sample task, and in a Lashley Type III maze. In shuttlebox avoidance conditioning, in which immunological disorder diminished performance, there was no difference between lesioned and sham animals. These results (1) support the dissociation between the effects on behavior of developmental neocortical anomalies and autoimmune disease, (2) point out similarities between spontaneous and induced neocortical malformations, and (3) fail to support behavioral differences between ectopies and microgyria.

This work was supported, in part, by NIH grant HD20806.

505.12 DIFFERENCES IN NEURONAL AND GLIAL MORPHOLOGY IN HUMAN SUBSTANIA NIGRA IN INTRA-UTERINE GROWTH RETARDATION (IUGR) AND COT DEATH CASES. J.C. van Velen, M.A. Brown, and E.Markova, Brain Research Institute, 5 Okbaha, 103604 Moscow, Russia & 'Dept of Fetal and Infant Pathology, University of Liverpool, L69 8)U, UK.

Free-floating sections of the mid-brains of 5 control, IUGR and cot death cases for each of three postnatal ages, 1 week, 4 weeks, and 4 months, were prepared for Golgi staining, Braingenberg modification. In addition to the 1 week group immune-histochemical stained for GAP-43 and TH. Qualitatively abnormal changes in Golgi stained dendrites, including varicosities, enlarged shafts of dendrites and aneurysm-like changes, were noted in the IUGR and cot death groups, which were similarly affected. Many neurons showed growth cone-like dendric endings and also filopodium-like thin dendrites arising from soma or proximal dendrites.

60 neurons from each case were measured, using a Leitz Orthoplan 3-D system. In both pathological groups a significant reduction in lengths of total dendritic tree, unbranched dendrites and terminal dendritic segments was revealed. In addition there was a reduction in the maximal radius of the dendritic trees in the IUGR and cot death groups compared to control.

A significant reduction in soma size of TH positive neurons with a concomitant increase in soma size of GAP-43 positive astrocytes was measured in IUGR and cot death groups compared to control.

The data suggests the presence of delayed maturation and pathological changes in SN neurons and a hyperexcitic astrocytic reaction, possibly associated with chronic hypoxic conditions during prenatal development. These results support the known clinical association between IUGR and cot death. It also may be relevant to findings of behavioral changes and impairment of cognitive function in groups of low birth weight infants.

EPILEPSY: ANTICONVULSANT DRUGS—AMPA AND NMDA RECEPTOR


Brain slices (350 µm) from the hippocampus of Long-Evans hooded rats were used. The control slices were incubated in ACSF, and all slices were incubated in ACSF containing 100 µg/ml valproic acid for 20 mins. After the treatment, slices were washed in Tris-HCl buffer, and prepared for PKC phosphorylation assay (Brain Res. 524 (1990) 144-148). Under the treatment of valproic acid, the cytosol PKC activity was 279 µg/mg/min (decreased about 35%); and the membrane PKC activity was 44 µg/mg/min (decreased about 27%). Valproic acid had a tendency to suppress PKC activity in both membrane and cytosol components.

506.2 SUPPRESSION OF REFRACTORY STATUS EPILEPTICS BY US4494A AND DIAZEPAM IN THE LITHIUM-PICOPIRINE MODEL IN RAT. A. Henderson, YMC West Los Angeles, Los Angeles, California 90073.

Treatment of clinical status epilepticus (SE) is frequently unsatisfactory; many cases are refractory to treatment or develop serious adverse events. We have utilized the lithium-picopirone SE model to investigate potential new therapies. In this model rats pretreated with LiCl (3 mg/kg ip) develop SE after picopirone is injected (30 mg/kg ip). Once ECoG-recorded continuous fast spiking is attained the SE is highly refractory to all conventional SE therapies.

US4494A has been shown to antagonize seizures in several models, but does not possess the sedative properties of the related kappa opioid agonist US4488H. We found that US4494A, 24-30 mg/kg, injected iv over 1 to 15 minutes (n=12) converted fast continuous spiking to periodic epileptiform discharges (PED). Higher doses that stopped all epileptiform activity with subsequent recovery of the rat (n=7). Diazepam administration after lower doses of US4494A that did not cause PEDs did not stop the (n=6). The combination of US4494A and diazepam thus is effective in stopping SE in this model of refractory SE. Supported by Upjohn.


AICI is a novel anticonvulsant drug that is efficacious in a wide variety of seizure models. AICI is a hybrid (a Na channel blocker and MK-801 an NMDA receptor un-competitive antagonist) and appears to have anticonvulsant activity consistent with both antagonistic activities. While the NMDA antagonist activities of this compound have been characterized electrophysiologically (Rogawski et al., J. Pharm. Exp. Ther., 1991) the direct evidence is available concerning AICI's activity as a Na channel blocker. In the present series of experiments, the sodium channel activity of AICI and its antagonists were evaluated using the whole cell patch-clamp technique in a cell line (NE1-115) in which TTX sensitive Na currents were isolated using the following recording conditions: external (in mM): 100 NaCl, 25 TEA, 1.8 CaCl2, 5 MgCl2, 1 MgCl2, 1 CoCl2, 25 glucose, 5 HEPEs, pH 7.35; internal (in mM): 150 ClO4, 4 MgATP, 0.6 GTP, 5 EGTA, 10 HEPEs, pH 7.35. AICI was found to block Na currents in a manner similar to that of carbamazepine. Both drugs were -5 fold more potent as blockers at -85 mV than at -85 mV. Similar to carbamazepine, AICI shifted the h, curve of the Na channels in a hyperpolarizing direction and showed a use dependent block of current amplitude. No differences were observed in the whole cell channel activity among (1AMDC) and its two (+ and -) enantiomers. 100 µM of the NMDA antagonist, -MK-801, had no detectable effect on Na currents. These findings suggest that the spectrum of anticonvulsant activity of AICI is not due to its interaction at TTX-sensitive Na channels in addition to its NMDA antagonistic activity.


Competitive AMPA receptor antagonists protect against convulsions and neuronal injury in animal models. LY293558 is (S-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid) receptor antagonist. In this study, the anticonvulsant and behavioral effects of LY293558 were evaluated following intravenous administration (1,3, or 10 mg/kg) in CD-1 mice. Convulsive thresholds for intracerebral administration of S-AMPA, electroshock and pentyleneetetrazol were dose-dependently increased. Clinical observations at higher doses included labored breathing (3 and 10 mg/kg), ataxia, hyperreactivity or hypoactivity (10 mg/kg). No overt clinical observations were noted at 1 mg/kg. LY293558 produced quantitative reductions in spontaneous and nonambulatory activity levels at 10 mg/kg. In addition, sensorimotor reactivity was decreased at the high dose of 10 mg/kg. Muscle tone and tremor in the hindlimbs were not affected by LY293558 treatment. These data suggest that LY293558 has anticonvulsant activity in several animal models.
EPILEPSY: ANTICONVULSANT DRUGS—AMPA AND NMDA RECEPTOR

**506.5**


Several studies showed that DM exerts both anticonvulsant and proconvulsant effects depending on the experimental conditions. In this study, we examined the effects of DM on KA-induced cell loss in the hippocampal CA3 region in vivo and in vitro. DM was given intraperitoneally (i.p.) to male rats before KA treatment, and the hippocampal CA3 region was examined for cell loss and ultrastructural changes induced by KA. DM antagonized the proconvulsant effects of KA, as evidenced by reduced behavioral abnormalities in the tail licking test. In vitro studies showed that DM protected neurons against KA-induced cell death, as demonstrated by reduced cell death and increased survival of cultured hippocampal neurons. These findings suggest that DM may have potential therapeutic applications in the treatment of seizures in rat models of epilepsy.

**506.6**

VALPROIC ACID, PHENOBARBITAL AND PHENYTOIN DO NOT DISPLACE \( [^3H] \)-MK-801 BINDING. J. M. Brown*, Dept. of Pharmacology, Northeastern Ohio University College of Medicine, Rootstown, Ohio 44272.

NMDA antagonists have anticonvulsant properties and are capable of enhancing the effects of other anticonvulsant agents (Urbanek et al., Eur. J. Pharmacol. 200; 1991; 277-282). Previous studies have demonstrated that the antiepileptic valproic acid, phenobarbital and phenytoin inhibit [\( ^3H \)] NMDA receptor binding in rat cortical homogenates in vitro. NMDA receptor antagonists are thought to interact with the NMDA receptor at a site common to all three receptor subtypes. However, the precise mechanism by which these drugs exert their anticonvulsant effects is not fully understood. The present study investigated the binding of [\( ^3H \)] NMDA receptor antagonists with and without these anticonvulsant agents in rat cortical homogenates. The results of these experiments demonstrate that valproic acid, phenobarbital and phenytoin do not affect [\( ^3H \)] NMDA receptor binding in rat cortical homogenates in vitro. These findings suggest that these drugs may act independently of the NMDA receptor to exert their anticonvulsant effects. Further studies are needed to determine the precise mechanism by which these agents exert their anticonvulsant effects.

**506.7**


MK-801 reduces the convulsant and anticonvulsant effects of kainic acid in mice. This study examined the effect of MK-801 on the regional expression of heat-shock protein-70 (HSP70), HSP70 mRNA and neuropsychological outcome following systemic kainic acid. HSP70 is a marker of cellular stress that does not invariably precede cell death. Suvorov and Lowenstein, 1992. Rats received either kainic acid (9 mg/kg), MK-801 (1 mg/kg) or MK-801 one hour prior to kainic acid. Kainic acid alone caused convulsions in 74% of treated rats. Convulsing rats expressed HSP70 mRNA and HSP70, predominantly in limbic regions. Nocice was also seen in select limbic areas. Pre-treatment with MK-801 reduced the percentage of convulsing rats to 4% and inhibited HSP70 mRNA and HSP70 expression mainly in thalamus and cortex. HSP70 mRNA and protein was maintained in CA3, subiculum and the amygdala. Rats pre-treated with MK-801 and not convulsing showed no histopathological changes. These data identify brain regions putatively involved in the manifestation of limbic convulsions and through which the anticonvulsant effect of MK-801 might be mediated.


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**506.8**


Remacemide (2-amino-1H-1,2-diphenyl-1,3-acetamidol) is a novel anticonvulsant drug that is metabolized to an open channel blocker of the NMDA receptor. Remacemide is also a weak NMDA receptor antagonist, but it appears to act at least in part, by a mechanism distinct from that of the metabotrophin. We have used several biochemical approaches to characterize the effects of cultured rat hippocampal neurons and [\( ^3H \)]idine binding to rat forebrain membranes to investigate the mechanism of the remacemide block. Remacemide inhibited NMDA-induced currents and displaced [\( ^3H \)]idine binding to rat forebrain membranes at a concentration of ~50 mM. The block of NMDA-induced currents at ~60 mV was weakly voltage-dependent and was reduced by ~15% at 0 mV. To estimate the voltage-dependent component of block, subsequent experiments were carried out at ~60 mV. Concentration-response curves for remacemide were obtained in the presence of absence of the NMDA agonist. Remacemide (100 mM) shifted the remacemide isotherm to the right (IC50 increased from 95 to 198 mM), whereas the purpored polyanamylate site antagonist diethylamidol (1 mM) produced a comparatively smaller shift (IC50 increased to 139 mM) using the rate of dissociation of [\( ^3H \)]idine as a measure channel activity, spermine (0.3-100 mM) was observed to markedly increase the rate of binding, whereas remacemide (10-100 mM) slowed the reaction. The concentra-

**506.9**

THE ANTI-CONVULSANT AND ANTICONVULSANT EFFECTIVE OF 2-NITRO-6-METHYL-7-CHLORO-2,3-DIMETHYL-1,3-QUINOXALINEDIONE (NMQX) AND 5-NITRO-1,3-DIMETHYL-2,3-DIQUINOXALINEDIONE (NDMQX), TWO NOVEL NMDA RECEPTOR/GLYCINE SITE ANTAGONISTS IN THE MES AND FORMALIN TESTS. M. I. Reza*, M. J. Latta, J. A. Reza, L. J. Reza, L. E. Reza, and E. W. Reza*. Acme Pharmaceuticals, Inc., Escondido Health Sciences Road West, Irvine, CA 92715; 2Dept. of Pharmacology, UC Irvine, Irvine, CA 92717; 3Dept. of Chemistry, University of Oregon, Eugene, OR 97403.

Recent studies have suggested a modulatory role for the N-methyl-D-aspartate (NMDA) receptor in mammalian psychological states, e.g., LTP, memory, learning, and depression. In the present study, we investigated the anti-convulsant and anticonvulsant effects of NMQX and NDMQX in the maximal electroshock (MES) and formalin test models in the female Sprague-Dawley rat. In the MES test, mice were injected i.p. with NMQX (1.25-7.50 mg/kg; N=4 mice/group) or NDMQX (5.0-15.0 mg/kg; N=4 mice/group). Control mice were injected with saline (0.05 ml) or dextrane (0.1 ml). Each compound was administered 30 min before MES. In the formalin test, following a 1 hr accommodation period mice were i.p. injected with NMQX (0.03-10.0 mg/kg; N=5 mice/group) or NDMQX (0.3-20.0 mg/kg; N=5 mice/group). Control mice were injected with saline (0.05 ml) or dextrane (0.1 ml). Each compound was administered 30 min before formalin injection. The results of these experiments demonstrate that NMQX and NDMQX are effective in the MES and formalin tests, and may have potential therapeutic applications in the treatment of seizures and pain.

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**506.10**


Felbamate, 2-phenyl-1,3-propanediol dicarboxilate, is a novel, orally active anticonvulsant that is reported to exhibit neuroprotective properties. Felbamate has recently been approved for the treatment of a variety of seizure disorders, including absence, myoclonic, and tonic-clonic seizures. The mechanism of action of felbamate is not fully understood. Felbamate appears to inhibit the spread of seizures and to elevate seizure threshold in animal models.

A proposed mechanism of action for felbamate is via the N-methyl-D-aspartate (NMDA) receptor complex. Neurotransmission mediated by the NMDA receptor complex has been shown to be associated with seizures, ischemic neuronal injury, and other neurological disorders. The neutral amino acid glycine, which acts as a co-agonist for the activation of the NMDA receptor channel, is postulated to be involved with seizure disorders and neuroprotection. Felbamate is demonstrated to have a limited ability of felbamate to inhibit binding at the glycine site on the NMDA receptor.

The present study examined the effects of felbamate on glycine-stimulated calcium ion influx with a minimal effective concentration of 100 nM. Dose response studies suggest a concentration-dependent relationship between felbamate and the reversal of calcium ion influx.

However, due to limited solubility, an extensive range of concentrations of felbamate could not be studied.

Felbamate (2-phenyl-1,3-propanedionedi carbamate) is a novel antiepileptic with a unique structure; however, its mechanism of action is unknown. Felbamate has been shown to bind to strychnine-insensitive glycine sites (McCabe et al., 1993) but thus far, this action has not been linked to its anticonvulsant effects. In the present study the effects of glycine on felbamate's ability to block convulsions was studied in two rodent models: N-methyl-D-aspartate (NMDA)- and electrostoch-induced convulsions. Briefly, NMDA (20 kmol/mouse) was injected i.c.v. and mice (CF-1) were observed for 30 min for clonic and myoclonic seizures. Electrostoch-induced seizures (50 mA, 60 Hz, 0.2 s through cerebel electrodes) produced tonic seizures in at least 95% of the mice. The ED50 for felbamate was shifted from 59 mg/kg to 705 mg/kg by pretreatment with glycine (800 mg/kg Ip.), with a potency ratio of 0.08 (p<0.05). An even greater shift was observed for blocking NMDD-induced convulsions. This interaction appears to be unique to felbamate since the ED50's for phenytoin, vigabartate, carbamazepine and phenobarbital were not increased by glycine. Plans to study newer anticonvulsants are underway. These findings suggest that felbamate blocks the actions of glycine and thus is the first anticonvulsant clinically available that acts by this novel mechanism.

507.1

Since several observations underscore the potential contribution of APP gene overexpression or dysregulated expression to Alzheimer's disease pathogenesis we judged important to elucidate molecular mechanisms of APP gene regulation at the transcriptional level and, as reported previously (Grilli et al. Society for Neuroscience, 1993, 421.8), we have identified a nucleotide sequence we refer to as APPKb in the APP gene regulatory region which specifically binds a protein complex belonging to the NFkB/Rel family of transcription factors. We now report that in transfection studies with constructs in which the APPKb sequence has been linked to a heterologous eukaryotic promoter (herpes simplex virus p promoter) governing expression of the bacterial CAT reporter gene, this nucleotide sequence behaves as a transcriptional repressor. The complex able to bind the APPKb sequence, unlike most of the NFkB/Rel family members, is constitutively present in nuclear extracts from various rat brain areas and cell lines we examined. It is feasible that this repressor protein may contribute to maintain low levels of APP gene expression and that at an alteration at any step in this regulatory pathway may result in deregulated expression of the APP gene. Future experiments will be directed to investigate alterations of this pathway in patients affected by Alzheimer's disease.

507.2
TWO NUCLEAR FACTOR BINDING DOMAINS ACTIVATE TRANSCRIPTION FROM THE HUMAN AMYLOID B-PROTEIN PRECURSOR (APP) PROMOTER. Wolfgang W. Quitteck* and James P. Matthews Department of Psychiatry and Behavioral Science, State University of New York at Stony Brook, Stony Brook, NY 11794-8101.

The promoter of the APP gene was analyzed for its ability to activate transcription in selected cell lines that showed variant levels of endogenous APP transcripts. Transient transfection studies showed that 94 base pairs 5' to the main transcriptional start site are sufficient for high levels of cell-type specific promoter activity.

Two nuclear factors that bind to this region in a sequence specific manner were identified by mobility shift electrophoresis, DNase footprinting, and methylation interference. One of the factors binds to the recognition sequence GGATACGTGAC, here designated as APB/PP. The other binding domain APBB contains the recognition sequence GCCGCTAGGGT.

The contributions of the binding domains APBPs and APBB to the activity of the APP promoter were assessed by introducing block mutations into the respective recognition sequences, which were analyzed by transient transfection. The results suggested that APBB contributes approximately 70-90% and APB8 10-30% to the transcriptional activity from the APP promoter in all cell lines analyzed.

507.3
AMYLOID PRECURSOR PROTEIN: LOCALIZATION OF mRNA AND PROTEIN ISOFORMS IN RAT OLFACTORY EPITHELIUM AND ALTERATIONS IN EXPRESSION DURING NEURONAL DEGENERATION AND REGENERATION. N. Zaidi and B. Talma*, Tufts Medical School, Boston, MA 02111.

The major component of the senile plaques in Alzheimer's disease (AD) is A4A or amyloid protein, which is derived from a larger integral membrane protein, Amyloid Precursor Protein (APP). A4A is toxic to neurons in culture and may contribute to neurodegeneration in AD. APP is widely distributed in neurons and other cell types, but the role of APP under normal physiological conditions and in AD is not understood. A single APP gene generates various transcripts by alternative splicing in both humans and rodents. APP 695 is the predominant form in rat central nervous system, while APP 751 and 770 forms (containing a Kunitz protease inhibitor (KPI) domain) are abundant in peripheral tissues. Recent studies suggest a role for APP 695 in neuronal differentiation; KPI-containing isoforms have been implicated in neuronal repair, regeneration and synaptogenesis. Olfactory neurons in nasal mucosa turn over throughout life at a slow rate, but accelerated turnover can be achieved by olfactory bulbectomy, thus providing an excellent model for examining the role of APP in neuronal differentiation and repair in adult animals in vivo. We have previously used RT-PCR and in situ hybridization with digoxigenin-labeled riboprobes to show that APP mRNA is localized primarily in the neuronal layer of the olfactory epithelium. Immunocytochemical studies using a C-terminally directed antibody that recognizes all full length isoforms show that APP is localized both in the neuronal layer of olfactory epithelium, and in nerve bundles. Experiments are in progress to localize APP isoforms by using isoform specific digoxigenin probes in control olfactory epithelium and during neuronal degeneration and recovery following bulbectomy.

507.4
CHANGES IN AMYLOID PRECURSOR PROTEIN AND RNA IN PRIMARY CULTURES OF EMBRYONIC CORTICAL NEURONS UNDERGOING GLUTAMATE-INDUCED NERVEDEGENERATION. D. Willoughby*, L. Rozovsky, and C. E. Finch. Andrus Gerontology Center and Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089.

Extracellular deposits of β-amyloid (Aβ) in disease-affected regions of the brain are a diagnostic feature of Alzheimer's disease (AD). Aβ is a 39-43 amino acid peptide derived from the amyloid precursor protein (APP). A Kunitz protease inhibitor (KPI) domain is encoded by an alternatively spliced exon of the APP gene. KPI-mRNA may be related to AD deposition or neuronal cell death in the AD brain.

To examine possible relationships between APP RNA alternative splicing and neuronal cell death, we measured alternatively spliced APP-mRNAs in primary cultures of rat embryonic cortical neurons treated with 250 μM glutamate. By LiDH release assay, glutamate-induced cell death was 2% after 4 hours of glutamate treatment, with variable cell death after 9 hours. Cell death was 45%, after 24 hours of glutamate treatment, KPI-mRNA was increased 2-3 fold after 6 hours of glutamate treatment, while APP-mRNA lacking KPI was unchanged. Aminocephamoleucinate, a NMDA receptor antagonist blocked glutamate-induced increases in KPI-mRNA. Thus, induction of KPI-mRNA was dependent on NMDA receptor activation.

Recently, it was shown by M. P. Mattson and co-workers that APP protected cultured rat hippocampal neurons from excitotoxic-induced cell death. We measured APP in cell lysates and conditioned media of neurons treated with 250 μM glutamate. APP was decreased in cell lysates of glutamate treated neurons within 1 hour of treatment. Cellular APP remained depleted for at least 20 hours following glutamate addition to the cultures. APP was also reduced in conditioned media 4 hours after glutamate addition. These results suggest that sustained glutamate receptor activation results in rapid degradation of APP. Induction of KPI-mRNA may be a compensatory response to glutamate-induced APP depletion in the neuronal cell, supported by AG0093 and AG7909.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
ACIDOSIS, NOT HYPOGLYCEMIA, STIMULATES PROCESSING OF BETA-AMYLOID IN CULTURED HIPPOCAMPAL NEURONS.


Reductions in cerebral glucose or increases in lactic acid in Alzheimer disease (AD) may signal pathophysiological mobilization of the amyloid precursor protein (APP). We tested these stresses of cellular metabolism on cultured rat embryonic hippocampal neurons over 4 days of growth in serum-free medium, either lactic acid was added to the medium or the medium was exchanged with a medium containing 20% fetal calf serum. After 24 hr, whole-cell and cell extracts were tested for immunoreactivity with antibodies specific for residues 1-42 of Aβ (426) or residues 425-594 near the C-terminus of APP69 (369kD). Hypoglycemia was found to have no effect on APP synthesis or processing. In contrast, lactic acid stimulated processing of APP. Less APP was secreted into the medium and more was found in the cytoplasm, processed to forms of apparent 21 and 25 kD. These forms were reactive with the antibodies directed against sequences for Aβ4 and the C-terminus of APP. Accumulation in the brains of the elderly may contribute to processing of APP that is potentially amyloidogenic. Supported by the Illinois Dept. Public Health.

MICROGLIAL PHAGOCYTOSIS OF PRE-AGGREGATED Aβ 1-42 AFTER INTRA-hippocampal INJECTION IN RATS. S.A. Johnson, R.L. Behel and C.E. Finch. Andrus Gerontology Center and Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.

Mature plaques in Alzheimer's disease brain are intimately associated with ameboid microglia that express surface markers consistent with antigen presentation and phagocytosis. EM studies showed intracellular amyloid fibrils in plaque-associated microglia, yet it is unclear if such intracellular amyloid was phagocytosed or degraded. Microglia in culture contain detectable APP mRNA, but in situ hybridization of AD cortex or hippocampus did not detect APP mRNA in plaque-associated ameboid microglia.

Since cultured microglia and fibroblasts accumulate exogenous Aβ 1-42, we examined the fate of Aβ 1-42 after intra-hippocampal injection in rats. Synthetic Aβ 1-42 was aggregated in PBS (1 mg/ml, pH 7.0, 48 hr, 25°C) with N-terminal biotinylated Aβ 1-42 (60:1:1) gift of Dr. C. Glabe) prior to stereotactic injection (tu). In pilot experiments, rats were sacrificed at 1 and 5d post-injection. Inset amyloid was visualized by avidin-biotin complex (ABC-HRP/DAAB histochemistry without antibodies, or by double-label histochemistry using nickel/DAAB to detect cell-specific antibodies.

We detected amyloid only at 5d post-injection. Double-label histochemistry showed bio-Aβ in Glial fibrillary acidic protein(184) positive macrophage/microglia. Combination ICC with anti-GFAP showed no intracellular accumulation of Aβ in astrocytes, but showed direct contact of astrocyte endfoot on extracellular Aβ. Numerous bio-Aβ-containing microglia (macrophage) surrounded the injection site. Some bio-Aβ-microglia were distant from the main Aβ 1-42 depot, occasionally surrounding, or even within, blood vessels, suggesting a route for clearance from the brain. This demonstrates microglial phagocytosis of extracellular, aggregated Aβ 1-42 in rat brain and suggests that microglia also phagocytose extracellular amyloid in the AD brain. Supported by AG10675 (SAJ) and AG07909 (CEF).

AN IN VIVO STUDY ON FACTORS AFFECTING AMINO ACID AND APP RELEASE FROM CORTICOSTRIATE NEURONS, USING PUSHER-PULL AND MICRODIALYSIS TECHNOLOGY.


A maintained inhibition on cortical pyramidal neurons through the 5-HT1A receptor contribute to the cognitive symptoms of Alzheimer's disease. In this study in the rat it was investigated whether a selective 5-HT1A receptor antagonist would enhance the activity of cortical pyramidal neurons, and whether an increased neuronal activity would stimulate secretion of APP. Striatal microdialysis and HPLC was used to assess aspartate and glutamate release as a marker of activity. APP in striatal pull perfuse was measured with Western blotting, using the N-terminal antibody 22C11. The selective 5-HT1A antagonist (+) WAY 100135 (50 μM) on the surface of the frontal cortex increased significantly basal glutamate release in the striatum, and potassium induced aspartate and glutamate release. Depolarization of striatal neurons with a potassium challenge through the pull pull probe induced an increase in 22C11 immunoreactivity. These results indicate that a selective 5-HT1A antagonist may be useful in attenuating the cognitive symptoms of Alzheimer's disease. In addition, as an increased secretion of APP may result in less amyloidogenic fragments being produced, an increased neuronal activity may also slow down the progression of the disease. Supported by the Brain Research Trust.


The post-ischemic time course of the expression of amyloid protein precursor (APP), apolipoprotein E (APO-E), glial fibrillary acidic protein (GFAP), and β-amyloid (β-AP), as well as the activation of microglia were examined immunocytochemically in the selectively vulnerable area of the hippocampus of gerbils subjected to 10 min of bilateral carotid occlusion-induced forebrain ischemia. These markers were compared to the time course of CA1 neuronal necrosis. Loss of CA1 neurons was detected between 3 and 72 hrs after ischemia, after which no further neuronal necrosis was observed. At 24 hrs postischemia, there was a decrease in APP, β-AP, APO-E, and GFAP in the CA1 region of the APO-E knockout mice. At 507.5 hrs, by 2 days, activated microglia were observed throughout the hippocampus. This coincided with a dramatic increase in the expression of APP, APO-E and β-amyloid between days 2 and 7. GFAP expression paralleled this increase, which is indicative of an activation of astrocytic protein synthesis. Reactive microglia were also observed in the CA1 region from days 4 to 7. It has been shown that the E isoform of APO-E, when observed, avoids binds to β-AP and, thus, increases the likelihood of β-AP deposition. Therefore, it is postulated that the increased expression of amyloid proteins coincident with an increased production of APO-E in response to ischemic neuronal necrosis provides conditions for the post-ischemic formation of amyloid deposits. Production of oxygen radicals by activated microglia may enhance the binding of β-amyloid to APO-E.

MICROGLIAL REMOVAL OF AMYLOID B PEPTIDE (AB) IS PREVENTED BY PROTEOGLYCAN. L.M. Shaffer*, M.D. Donley, S.G. Younkin and K.R. Brandt. GliaTech, Inc., Cleveland, OH 44123, and Case Western Reserve Univ., Dept. of Pathology, Cleveland, OH 44106.

In the Alzheimer's Disease (AD) brain, activated microglia are found closely associated with the core of mature neuritic plaques. The composition of the plaque is Aβ 40-42 amino acid peptide which aggregates to form fibrillar extracellular deposits. We have previously demonstrated that primary cultures of rat microglia and a human microglial cell line, GM1, are capable of removing Aβ under conditions where the fibrillar peptide was in solution or immobilized on a substrate, mimicking a plaque-like structure. There is thus a question of why plaques in the AD brain persist in close proximity to activated microglia. This persistence could result from the association of Aβ with other plaque components, such as, inactivating it in vivo and removal mechanisms. Since proteoglycans are inviable feature of all amyloidosis and are known to bind Aβ, they may contribute to plaque formation and persistence by altering microglial proteolytic ability. We have determined whether proteoglycans could prevent removal of Aβ by rat microglia and the human monocyte line, THP-1. Using zymo-vass-T staining to detect immobolized Aβ before and after treatment, it was found that both cell types showed good adhesion to the peptide substrate and removed the Aβ in approximately one week. Because astrocytes are a likely source of brain proteoglycans, primary rat astrocytes were grown in culture dishes containing immobilized Aβ to allow secreted molecules to associated with the peptide. We found that removal of the astrocytes and subsequent seeding of microglia or THP-1 cells, the conditioned Aβ was found to be resistant to breakdown. Direct application of proteoglycan purified from astrocytes onto the Aβ substrate also resulted in inhibition of microglial processing of the peptide. These data suggest that astrocyte-derived proteoglycans may play a role in the persistence of Aβ.


It is established in the literature that cholinergic stimuli increases APP secretion in a variety of neural and non-neural cell lines transiently expressed to the M1 and M3 muscarinic receptors. Occupation of M1 and M3 receptors results in phosphorylation via a G-protein. These data have led to the theory that APP metabolism is controlled, at least in part by neurotransmitter receptors. Therefore, we provide evidence that for the muscarinic control of APP processing in NT2N cells, which may provide a unique model system for understanding the contributions of neurons to amyloidogenesis. Previous studies, we have provided evidence that the NT2N cells expresses the 695 amino acid APP isoform and secretes intracellularly produced APP peptides. We now demonstrate that NT2N cells express similar levels of endogenous M2 and M3 receptors as well as the GTP-binding protein, Gq. Carbachol, an M1 and M3 agonist, induces the activation of phospholipase C in NT2N cells. Pretreatment with the EIPA, or in the levels of the second messenger end-products diacylglycerol and inositol triphosphate (IP3) which peaked at two min. Subsequently, as would be expected from the increased diacylglycerol in the cells, the levels of IP3 and PIP2, were increased in the levels of the second messenger end-products diacylglycerol and inositol triphosphate (IP3) which peaked at two min. Subsequently, as would be expected from the increased diacylglycerol in the cells, the levels of IP3 and PIP2, were increased.
PKC STIMULATION BUT NOT CHOLINERGIC RECEPTOR STIMULATION INCREASES THE PRODUCTION OF THE AMYLOID PRECURSOR PROTEIN (APP) IN CULTURED RAT CORTICAL NEURONS. J.L. Mills and P.B. Reiner

Kensington Laboratory of Neurological Research, University of British Colombia, Vancouver, B.C., Canada V6T 2E6.

The progressive deposition of the amyloid b-peptide (Aβ) is an early and invasive feature of Alzheimer’s disease pathology. The Aβ sequence is contained within a much larger precursor, called the amyloid precursor protein (APP) which can be processed by multiple proteolytic pathways. Converging secretory processing of APP is thought to be nonamylodigenic, since cleavage occurs within the Aβ sequence, resulting in soluble secretion of APP. Alternative processing routes are potentially amyloidogenic yielding the Aβ sequence intact.

PKC signal transduction pathways have been implicated in the regulation of APP secretion. These data are derived from studies in continuous cell lines. It is not known whether or not this same mechanism of control occurs in central neurones. In the present investigation, we examined whether direct or indirect stimulation of PKC increases the secretion of APP in primary cultures of rat cortical neurones. Activation of PKC by the phorbol ester phorbol 12,13-dibutyrate (PDBu) or phorbol 12-myristate 13-acetate (PMA) significantly increased the release of APP. In contrast, activation of the PLC/PKC-coupled muscarinic m1 receptor by oxotremorine-M did not significantly increase basal release of APP. Similarly, chemically induced depolarization using 35 mM KCl did not alter APP secretion. Our data indicate that although PKC stimulation can alter APP processing in central neurones, neuronal activity and cholinergic neurotransmission does not.

PROCESSING OF THE AMYLOID PRECURSOR PROTEIN WITH THE THIOGLYCERALDEHYDE REACTION IN THE SECRETORY PATHWAY. R.G. Perez and E.H. Koop, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA 02115.

A mutation at codon 670/671 of APP (KM → NL) from one form of familial Alzheimer’s disease results in significantly elevated Aβ release by an undefined mechanism. Because we have recently demonstrated that Aβ production and release involves the internalization and recycling of cell surface APP, we asked whether the codon 670/671 mutation alters APP processing in this pathway. As expected, Aβ release was significantly elevated in CHO-K1 cells transfected with KM → NL mutation (6NL), as compared to APP751 WT (WT) cells. However, the kinetics of Aβ release was different in 6NL as compared to WT cells such that Aβ release occurred earlier in 6NL cells. The enhanced release of Aβ in 6NL cells was due to an intracellular 12 kDa C-terminal APP fragment. In addition, a lower molecular weight APP species was present intracellularly and released into medium of 6NL cells. By epitope mapping, this shorter APPs from 6NL cells is consistent with a "b-secretase" cleaved product. The premature intracellular cleavage of APP by b-secretase resulted in a 50% reduction of full length APP sorted to the cell surface. Moreover, Aβ release was not significantly elevated in 6NL cells after cell surface labelling, demonstrating that although Aβ generation also occurred after internalization, in 6NL cells, processing of APP was not altered in this pathway. In sum, our studies demonstrated that the KM → NL mutation resulted in a selective alteration of APP processing by b-secretase. More importantly, our results suggest that the early cleavage of APP at the b-secretase site occurs in a different compartment within the secretory pathway in 6NL cells.


The growing interest in the identification, isolation and inhibition of secretases which generate the amyloid b-peptide from APP necessitates the production of purified recombinant substrates for secretases. These purified polypeptides will be useful tools for studying physiological and pathological functions of APP, and for the identification and characterization of secretases involved in Aβ peptide formation.


Secretory cleavage of the amyloid precursor protein (APP) of Alzheimer’s disease (AD) is stimulated by the muscarinic agonist carbachol in human embryonic kidney (HEK) cells expressing human amyloid b-amyloid precursor protein (BAPP) which can be processed by multiple proteolytic pathways. Converging secretory processing of BAPP is thought to be nonamyloidogenic, since cleavage occurs within the Aβ segment, resulting in soluble secretion of BAPP (APPs). Alternative processing routes are potentially amyloidogenic yielding the Aβ segment intact.

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THE INCREASES IN BAPP, puriﬁed from conditioned medium of APP expressing human embryonic kidney (HEK)-293 and BAPP-expressing human embryonic kidney (HEK)-293 cells after 24 hrs treatment with a combination of cholinergic agonists (carbachol) and a PKC activator (PMA) was determined. This result was conﬁrmed by immunocytochemistry using a cholinergic receptor antibody. This process was mimicked by staurosporine, a PKC activator. However, a more selective PKC inhibitor, G10920X, completely blocked the response to phorbol esters, but not partially inhibited by (SO2) the increase in the release of soluble derivatives of APP (APPs) elicited by carbachol. Since staurosporine also inhibits proteins tyrosine kinases, we investigated the involvement of tyrosine phosphorylation in the regulation of APP release. Carbachol increased tyrosine phosphorylation of a number of proteins in HEK cells, and this effect, like APPs release and PKC activity, was preferentially coupled to m1 and m3 receptors. Moreover, an inhibitor of protein tyrosine phosphatase (vanadyl hydroxide) prevented APPs release. The result suggests that both PKC and protein tyrosine phosphatase contribute to the immunocytochemical APP processing by muscarinic receptor activation. (Grant #MH67835 of NIMH)


Cathepin S is a cysteine lysosomal protease resistant to neutral pH. Our studies in rat brain as well as peripheral tissues have shown that it is preferentially expressed in cells from the mononuclear phagocyte lineage. Of all cell lines tested so far cathepin S transcript could be detected only in monocytic leukemia cell lines. Studies on enzolial cortex lesions in rat brain have shown an upregulation of expression of cathepin S in activated versus resting microglia, suggestive of an important role for this protease in responses of neuronal degeneration and regeneration.

We have studied the changes in the level of cathepin S transcripts that occur upon differentiation of the HL 60 leukemia cell line. The possibility of cathepin S to be secreted in the media of normal leukemia cells, differentiated leukemia cells as well as macrophages challenged with inflammatory agents was also assessed.

Cysteine lysosomal proteases have been implicated to take part in the metabolism of the Amyloid Precursor Protein (APP). The fact that this cathepin S is expressed in microglia in brain, a cell type pertinent to the pathology of Alzheimer’s and other neurodegenerative diseases, its resistance to neutral pH and its substrate specificity (preference for small neutral amino acids N- terminal to the cleavage bond), makes cathepin S a strong candidate for a protease involved in the generation of the Aβ peptide from the APP. On the other hand, other research groups have shown that secreted media from microglia are able to cleave soluble and aggregated Aβ peptide.

Here we present the processing pattern obtained by in vitro proteolysis of the wild type and mutant variants (Swedish and Dutch) of the full APP by cathepsin S. The ability of cathepsin S to degrade soluble and/or aggregated Aβ peptide is also addressed. Supported by NS26580 (L. D.).

THE LOCALIZATION AND INTERNALIZATION OF CELL SURFACE BAPP IN CULTURED NEURONS. 2. Yamazaki, D.J. Silkeas and E.H. Koo*, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA 02115.

At the cell surface, the b-amyloid precursor protein (BAPP) may be released after cleavage by n-secreterase or internalized without clearance. Recent evidence suggests that the latter pathway may be important in the constitutive production and release of amyloid b-peptide. Using monoclonal antibodies that recognize the extracellular domain of BAPP, we previously demonstrated that cell bound BAPP in CHO cells is internalized via coated pits and targeted to lysosomes. In this study, we demonstrate that in primary neurons, BAPP is internalized into a lysosomal compartment. The length BAPP is similarly located on the cell surface and subsequently endocytosed. BAPP was present on the cell surface of neurons cultured at low density shortly after plating (Bampkin 1). Immature neurons (Stage 3) showed a discrete compartment and BAPP immunostaining on the soma. On axons and minor neurites, BAPP staining was distributed in a distinctly segmental pattern. Immature neurons (Stage 5) showed remaining discontinuous and was located principally on axons. BAPP immunoreactivity was colocalized with a1, a5, and B integrins, but not with NCAM, synaptophysin, transferrin, or neurofilament. In mature neurons, the internalized BAPP was located in a compartment which contained fluid phase endocytic markers. This staining pattern was also seen in both the perikaryal and neuritic processes. In mature neurons, internalized BAPP was located principally in axons and soma. To demonstrate endocytosis of BAPP from axonal surface, dissociated sympathetic neurones were grown in compartment (Campyon) cultures. Using this system, BAPP was clearly internalized from the cell surface of distal axonal sites and retrogradely transported to the neuronal soma. In summary, these studies in primary neuronal cultures show that 1) in early stages, cell surface BAPP was not distributed in a polarized manner, 2) in mature cultures, surface BAPP was localized predominantly at the axonal site and 3) in mature neurons, BAPP was retrogradely transported from the surface of distal axonal sites to the cell body.
507.17

DEGENERATIVE DISEASE: ALZHEIMER'S—BETA AMYLOID VII

507.18

NEURONS DERIVED FROM A HUMAN TERATOMA CELL LINE GENERATE INTRACELLULAR Aβ PRIOR TO ITS SECRETION INTO CONDITIONED MEDIUM R.S. Turner* and Y.M.-Y. Lee, Dept. of Neurology and Pathology and Laboratory Medicine, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104-4280.

Alzheimer's disease (AD) brain is characterized pathologically by neuronal and synaptic loss, neurofibrillary tangles which consist primarily of the microtubule-associated protein tau, and amyloid plaques which consist primarily of a 42-kDa peptide (Aβ) derived from one or more of at least three alternatively spliced amyloid precursor proteins (APP). We examined the kinetics and intracellular localization of APP metabolism in cultured neurons derived from a human teratocarcinoma cell line (NT2). These neurons constitutively secrete a 95-kDa fragment of APP (APPs) and Aβ. Metabolic labeling of NT2 neurons with [3H]leucine followed by immunoprecipitation of APP, APPs and Aβ from cell lysates and conditioned medium revealed that APP was first recovered from cell lysates and, after an approximate 2 hour delay, from conditioned medium. In addition, cellular Aβ increased overnight while Aβ in conditioned medium continued to increase. This suggests that APP is first generated intracellularly soon after APP synthesis and then secreted into medium. In the presence of 10 μM limiting chelator, cellular APP accumulated with no evidence of its metabolism, thus precluding the secretion of APP fragments into medium.

507.19


Human amyloid protein precursor (APP770) and its carboxy terminal portion (CT180) including β/α-3 domain were highly expressed in an E. coli system. The in vivo recombinant APP peptides were purified with a combination of urchin solidification and ion-exchange chromatography and used for proteolytic processing by thrombin. Three thrombin cleavage sites were predicted by the decrease of isoelectric point and the appearance of K56, 27 and a K45 fragments containing β/α-4 domain on SDS-PAGE gel and on the immunoblot. The carboxy-terminal proteolysis of plateaued APP expressed to thrombin resulted in the stimulated production of 60 and 27 kDa carboxy-terminal peptides containing the intact β/α-4. This thrombin-mediated proteolysis was catalyzed by the specific thrombin inhibitor. These results suggest that thrombin may play a role in APP-cleaved peptide precursors amyloidogenic intermediates in vivo leading to amyloid deposition.

507.20

EXPERIMENTAL CHLOROACETATE MYOPATHY IN RATS—MODEL FOR APP PROCESSING.


1Dept of Microbiology, 2Dept of Neuropharmacology, Sapporo Medical University, 3Dept of Psychology, Hokkaido University, Sapporo, Japan.

Deposition of β/α protein is most characteristic pathological change in Alzheimer's disease. There have been no good models for studying β/α production. Chloroacetate, a pyridoxine antagonist, induces muscle pathology in experimental and human disorders. Chloroacetate is a compound with toxicological properties similar to human β/α protein to induce muscle pathology, in which β/α deposition is observed. In this study, we present morphological, immunopathological, and biochemical data in regard to APP processing in this experimental model.

Isolated and denervated soleus muscle from chloroacetate treated and untreated WKA rats were studied. Atrophy of muscle fibers, numerous dense granule bodies, and vacuoles were seen in denervated soleus muscle of chloroacetate treated rats. Progressive destruction of muscle, and proliferation of connective tissue were predominant in WKA rats. Denervated soleus muscle stained with anti-cathapsin D, and several anti APP antibodies. Western blot analysis showed that 49, 50 kDa bands and 20 kDa bands were detected in buffer soluble, and detergent soluble fractions with anti β, 25, and anti C terminal antibodies respectively in chloroacetate treated rats.

This experimental model seems to be useful for study of APP processing. And present data suggests lysosomal pathway is involved in β protein production.

507.21

APP EXPRESSION IN THE CELL LINE NT1A2. R.B. Watson, P.R. Harris, P.W. Andrews, and E.C. Pearson, Department of Biochemistry, The University of Sheffield, Western Bank, Sheffield S10 2NY, UK.

The cell line NT1A2 is a useful tool for studying the mechanisms of protein processing with relevance to human neurodegenerative disease (Andrews, 1984; Wurtkin et al, 1993) particularly the differential expression of the mRNA encoding for Amyloid Precursor Protein (APP) in neurotrophic factor (NTF) rich work (Adkerman et al, 1991) has shown using cDNA libraries that APP105 and APP73 are present in undifferentiated and differentiated cell lines. APP73 was observed in libraries from differentiated, neurite-like cells only.

The study used as a new hybridization histochemistry to examine the occurrence of APP mRNA transcript and Western blotting to examine the relative isoforms produced in the NG108-15 cells. Hybridization probe for APP105 was used to probe the mRNA sequences for APP105 and APP73, a 2800 bp cDNA clone. Probenes were used to correspond to regions encoding the β/α sequence (β/α-3), to a sequence specific to APP73 (APP105, APP670), to some APP105, APP750, APP730, APP530, and APP420. APP105 was observed in libraries from differentiated, neurite-like cells only.

The study used as a new hybridization histochemistry to examine the occurrence of APP expression of APP105 and APP73, a 2800 bp cDNA clone. Probenes were used to correspond to regions encoding the β/α sequence (β/α-3), to a sequence specific to APP105 (APP105, APP670), to some APP105, APP750, APP730, APP530, and APP420. APP105 was observed in libraries from differentiated, neurite-like cells only.

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DEGENERATIVE DISEASE: ALZHEIMER'S—BETA AMYLOID VII

508.1 CEREBRAL AMYLOID ANGIOPATHY IS ASSOCIATED WITH CORTICAL NEURONAL LOSS. C. Zampi, B.A. Limousin, H. Chui. Department of Neurology, University of Southern California, Rancho Los Amigos Medical Center, Downey, CA 90242. The deposition of B-amyloid in cerebral blood vessels (so-called cerebral amyloid angiopathy (CAA)) is a frequent finding in Alzheimer disease (AD). An association between CAA and cerebral hemorhage and infarction is well-recognized, but potential ongoing effects of CAA on neuronal survival are not known. We examined the relationship between the severity of CAA and numbers of neurons in five neocortical areas and entorhinal cortex in 103 neuropathologically-confirmed cases of AD. CAA was rated on a semi-quantitative scale (0-3) in a thionine stained leptomeningeal and cortical vessels. Neurons, neurofibroblastic tangles, and senile plaques were counted in complete cortical columns in semi-adjacent sections stained with cresyl violet or Bielschowsky silver method. Some degree of CAA was found in at least one region in 87% (84/103) of cases. Severe CAA (grade 3 in at least one region) was found in 29% (30/103) of cases. Severity ratings for leptomeningeal and cortical CAA were highly correlated. The correlation coefficient was 0.79. The relationship between the severity of CAA and neuronal number in area 22 (r = 0.22, p = 0.0005) and the severity of leptomeningeal CAA and neurons in area 9 (r = 0.21, p = 0.048). The relationship between the severity of cortical plaques, senile plaques, and age at onset and neuronal number was further examined using multiple regression analyses in each anatomical region. Again, cortical CAA in area 22 (p = 0.007) and leptomeningeal CAA in area 9 (p = 0.02) were significant predictors of neuronal loss. We hypothesize that CAA may compromise blood flow and the delivery of essential nutrients to neurons; alternatively, B-amyloid may exert a direct neurotoxic effect. (NIH 2P50AG01424 and AG07624)

508.2 IN VIVO EFFECTS OF THE AMYLOID B PROTEIN IN THE RAT BRAIN. P.J. Action and D. Ghetti. Indiana Univ. Sch. of Med., Terre Haute Center, Terre Haute, IN 47809 and Indiana Alzheimer Disease Center, Indianapolis, IN 46202. The specific aim of this study was to compare the long-term in vivo effects of senile plaque cores (SPC) or synthetic amyloid b peptide (Ab) in the rodent brain at 2 different ages. SPC-enriched pellets were isolated from the temporal cortex of Alzheimer brains. Synthetic Ab (1-40) was purchased from Bachem, Philadelphia, PA, dissolved in phosphate buffered saline at a concentration of 50 µM and incubated at 37°C for 48 hrs prior to use. Pressure injections were performed in the cortex of young (6 months) and old (20 months) male Fischer 344 rats and the animals were allowed to survive 1 or 3 months. At the end of the survival periods, the rats were euthanized and perfused. The brains were processed for histology and immunohistochemistry. Hypersia and hypertrophy of astrocytes surrounding the injection site were observed at up to 5 months in all cases. The injection sites were clearly seen at 1 and 3 months due to cavitation and deposition of the injected SPC or Ab. These deposits were strongly immunoreactive to Ab antisemur at 1 month but were less so at 3 months. ALZ50 immunoreactivity, which is indicative of abnormal cytoskeletal changes, was seen and around the injection site at 1 month and was substantially decreased at 3 months. No major difference was seen between the use of SPC or Ab, or between young or old animals. Thus, over a long survival period, acute in vivo injection of SPC or Ab in the rat brain results in clearance of the injected deposits and attenuation of abnormal cytoskeletal changes. Supported by grant #P01AG10133.

508.3 HISTOLOGICAL AND BEHAVIORAL EFFECTS OF INTRAHIPPOCAMPAL INJECTIONS OF B-AMYLOID PEPTIDE (B-AP) IN THE MALE RAT. A.W. Doman, A. W. Bordon, E.N. Korelits, A. R. V. McCampbell, A. R. Peterson, G. P. Tinker. Dept. of Psychology, Illinois Wesleyan Univ, Bloomington, IL 61701. Neuroscience Dept, Abbott Laboratories, Abbott Park, IL 60064; Veterans Res Educ Clin Ctr. Veterans Admin Med Ctr., Bedford MA 01730. Alzheimer’s disease is characterized by the extracellular deposition in the brain of insoluble aggregates of the amyloid b peptide (Ab). Evidence from several laboratories has demonstrated the neurotoxicity of Ab in vitro, and in vivo. Few studies, however, have examined the behavioral effects of Ab when injected into the rat brain. In a series of experiments, a comparative histological and behavioral assessment were conducted following injections of Ab(1-42) into the hippocampus and medial septal area of male rats. Experiment 1: animals received bilateral injections into the hippocampus of 3.5 nmol/SL of either Ab(1-42), CA(1-42) in a scrambled form, or DM50. In experiment 2, animals were injected bilaterally into the medial septal area with 3.5 nmol with Ab(1-42), the scrambled sequence or DM50. In experiment 1, animals were tested on a radial arm maze. In experiment 2, 2 animals were tested following injections of Ab(1-42) using the Morris Water Maze. Following the behavioral tests, animals were sacrificed and analyzed for CA1 activity in the hippocampus either 7 or 14 days following injections. In experiment 3, another group of animals were injected with 3.5 nmol Ab(1-42) into the medial septal area or hippocampus. Following a two week survival period, animals were sacrificed and their brains were processed for immunocytochemistry with Ab antibody. Our preliminary results indicate that bilateral injections of Ab(1-42) into the hippocampus or medial septal area had no significant effect on spatial learning in the male rat. Additionally, no significant differences were found in CA1 activity activity in the hippocampus following injections of Ab into the septum at 7d controls (15.877 ± 0.371 pmol/min/g ptt; Ab = 15.65 ± 0.78 pmol/min/g ptt) or at 14 d. The histological study in in progress, and the results of this study will also be presented at this conference.

508.4 COMPARISON OF THE EFFECTS OF INTRAHIPPOCAMPAL INJECTIONS OF A-(1-42) IN COMBINATION WITH GLUCOCORTICOIDS AND IBOTENIC ACID ON THE ACQUISITION AND RETENTION OF A SPATIAL TASK IN MALE RATS. W.A. Doman, A. J. Giovannetti, A.B.Y. McCampbell, H. K. Witegwer, J.S. Puccio, L.J. Chapman, S.M. Bond, A.R. Peterson, J.H. Hickman. Dept. of Psychology, Illinois Wesleyan Univ, Bloomington, IL 61701. Alzheimer’s disease is characterized by the extracellular deposition in the brain of insoluble aggregates of the amyloid b peptide (Ab), a 39-43 amino acid peptide. Although the cause of neuronal degeneration in AD remains unclear, one major focus is on the role of BA. Evidence from several laboratories have repeatedly demonstrated the neurotoxicity of BA in vitro, however, other investigators have reported little direct neurotoxicity, but rather an increase in the vulnerability of neurons to EAs. Furthermore, few studies have examined the behavioral effects of Ab when injected into the rat brain. In a series of experiments, we assessed the effects of bilateral injections of BA(1-42) 5nmol/SL into the hippocampus on both acquisition and retention of a spatial task in the male rat. In experiment 1, intact or adenorexaminized animals were injected with either BA(1-42) or a scrambled sequence of BA with either daily injections of Tmgl/mL corticosterone at a concentration previously reported to induce artificial stress levels as assessed (controls). In experiment 2, animals were injected with either ibotenic acid (Hemato/PL) and corticosterone and BA(1-42) in a scrambled sequence; or IBO in combination with BA(1-42). Animals in experiments 1 and 2 all animals were pre-trained on a radial arm maze task with 5 of 8 arms baited. In group 1, half of the animals were pre-trained, the other half were tested for acquisition. After surgery the animals were tested for two weeks with the configuration preserved. Animals were tested for 2 weeks with the new configuration. A variety of behavioral parameters were measured using the radial arm maze. Our preliminary results indicate that there is a clear disruption of learning performance in animals that received BA(1-42) in combination with corticosterone.
DEGENERATIVE DISEASE: ALZHEIMER'S—BETA AMYLOID VIII


It has been suggested that the complete neurotoxic activity of BAP 1-40 may be contained in the fragment BAP 25-35. In this study, LPS-induced, multiple intrahippocampal injections of BAP 25-35 impaired retention of one-trial reward learning 2 weeks later. The present study investigated whether similar injections would cause such severe, long-lasting degenerative changes that the task could not be retained, even if training began 4 weeks after injection. If LPS was employed to reveal aging and modulation of cholinergic mechanisms. The subjects were 39 male rats, 3-4 months old. They received 7 hippocampal injections of 6 nmol BAP 25-35 (n=21) or distilled water alone (n=18), bilaterally. After recovery and deprivation to 82% of initial weight, they received an initial training trial 28 days post-injection, and 3 subsequent daily retention trials. The apparatus was a sunburst maze with 4-level alleys and 1 bated ascending alley with a grid floor. Rats were scored for errors (entrances into unbaited alleys). Each rat's retention score was its decrease in errors relative to the baseline trial. The control rats steadily reduced their errors from day to day, whereas the BAP-treated rats showed little improvement. Tukey's HSD procedure revealed significant, p<.05, differences between treatment groups on the last 2 retention trials.


It has been suggested that the complete neurotoxic activity of BAP 1-40 may be contained in the fragment BAP 25-35 and that pre-incubation of BAP 25-35 might increase their aggregation and neurotoxicity. Therefore, multiple intrahippocampal injection of BAP 1-40 impaired retention of learning that occurred 2 weeks later. In this study, 3-4 month-old rats received 7 hippocampal injections per side of 6 nmol BAP 25-35 (n=14) inoculated for 24 h at 37°C or non-incubated BAP 25-35 (n=3) inoculated 48 h after inclosure (n=3). After deprivation to 82% of initial weight, they received an initial training trial 14 days post-injection, and 3 subsequent daily retention trials. The apparatus was a sunburst maze with 4 level alleys and 1 bated ascending alley with a grid floor. Each rat's retention score was its decrease in errors (entrances into unbaited alleys entered) from the baseline trial. Retention scores, averaged across 3 trials were 5.4±1.4 (SEM) for the control group. For the incubated BAP group and for the non-incubated BAP group. ANOVA indicated a significant, p<.05, drug effect and post-hoc analysis (Tukey's HSD) indicated that only the non-incubated BAP group was significantly different, p<.01, from controls. One-sample t-tests indicated significant retention in all groups except non-incubated BAP.

080.7 EVALUATION OF SPATIAL LEARNING IN Rats WITH INTRAHEMISPHERIC INFUSION OF BAP. TATE, D. Rocas, R. E. Malocha and C.A. Marotta. Dept. of Psychiatry and Human Behavior, Miriam Hospital and Dept. of Neuroscience, Brown University, Providence, RI 02906.

A chronic intraventricular β-amyloid infusion model was used to assess cognitive and memory deficits. During infusions of either β-amyloid peptide (1-40) or glucose vehicle vehicles, male rats were tested in a spatial memory task. Animals were required to locate a hidden platform to escape from a pool of water. After 2-4 days of testing, the amyloid-infused rats had significantly longer lattencies to escape than glucose infused rats, but eventually reached latencies equal to control animals. After four days of testing, the platform was removed and the amount of time spent in each quadrant of the pool was measured. Only the glucose-infused animals spent significantly more time in the quadrant that previously contained the platform. Therefore, amyloid-infused rats showed subtle memory deficits. When animals were sacrificed, immunostaining of the brains revealed β-amyloid-positive deposits and significant glosis in the brains of amyloid-infused rats.

080.8 INFLUENCE OF AMYLOID PRECURSOR PROTEIN ON TROPHIC RESPONSE OF PC12 CELLS TO NEUROTROPHIC FACTOR. R.B. Malocha, S. Agrawal, E. Humke, J. Yang and C.A. Marotta. Dept. of Psychiatry & Human Behavior - Neuroscience, Brown University and Miriam Hospital, Providence, RI, 02912; and, Hybriedon, Inc., Worchester, MA 01605.

While the relationship between NGF and the amyloid precursor protein (APP) is of interest with respect to the potential therapeutic potential of growth factors applied to Alzheimer's disease, changes in APP levels and distribution in PC12 cells treated with NGF have not been reported. To explore the relationship between APP and NGF, we applied antisense oligonucleotides (AO), containing a phosphorothioate backbone, complementary to the 5’ and 3’ end region of APP mRNA, to stimulated and unstimulated PC12 cells. APP AO significantly reduced the level of APP compared to the non-nucleotided AO. Maintaining APP AO in cultures (2µM, 48hr) both prior to and during NGF addition increased retention of cell size and neurite extension, as compared with controls. Since reduction of APP levels by AO reduced the response of PC12 cells to NGF, APP may be a modulating element of the trophic factor cascade.


The protein products of the Jun and fos immediate early gene (IEG) families are cooperative transcriptional regulatory factors which act as cellular 'third messengers' and have been implicated in regulating the expression of many genes, including the amyloid precursor (APP). We have previously demonstrated that immunoreactivity for Jun and fos-related proteins is induced in the hippocampal dentate gyrus and associated with neuronal pathology (Exp. Neurol. 125: 286-305), that these proteins are induced by Aβ in cultured hippocampal neurons, and that Jun proteins are induced in DA-102-7E rat neurons that are resistant to Aβ-induced cell death (submitted). Several of these genes have been implicated in the control of apoptosis. As a result, we have suggested that the expression of some IEGs (e.g. one or more of the Jun proteins) may be related to or participate in Aβ-induced apoptosis, and that the expression of Jun proteins is an early event in Aβ-induced neuronal death. We have speculated that a variety of Jun proteins indicates that Aβ induces a sustained increase in Jun proteins in culture, whereas other agents which induce IEGs such as phorbol esters produce a more transient and typical expression in Jun protein expression. Immunoblotting with a specific antibody to Jun B shows a similar pattern of Aβ-induced Jun immunoreactivity, but do not provide evidence for the induction of Jun B in response to Aβ. These data suggest some specificity in the cellular response to Aβ, which should be apparent in the regulation of these genes at the mRNA level.

080.10 EFFECTS OF α2-ANTICHYMOTRYPsin ON THE TOXICITY OF β-AMYLOID FRAGMENT 25-40 IN RAT PRIMARY CULTURED NEURONS. N. Nishiyama*, T. KOBAYASHI and H. SAITO. Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., The Univ. of Tokyo, Tokyo 113, JAPAN.

B-Amyloid is the main component of senile plaques. Neurotoxic effects of β-amyloid peptide has been suggested to be involved in the pathogenesis of Alzheimer's disease (AD). We used a novel synthetic peptide β25-35, corresponding to the presumed presumable toxic domain, to test its effect on neuronal cells. α2-Antichymotrypsin (ACT), a serine protease inhibitor, colocalizes with amyloid deposits. ACT might contribute to the development of amyloid deposits and pathogenesis of AD through its protease inhibitory activity. We investigated the combination effects of ACT with a novel β25-30 on rats' primary cultured neurons. Single cell suspensions of hippocampus and cerebral cortex were prepared from fetal rat brain, and plated at either high (1x10⁴ cells/cm²) or low (5x10³ cells/cm²) cell density. Desired concentrations of ACT were added to the medium. The cells were visualized by cresyl violet staining or MAP2 immunohistochemistry. In some cases, cell viability was evaluated by MTT assay. Although ACT (0.1-10 µg/ml) did not affect the neuronal survival in high density culture, it decreased the number of surviving neurons concentration dependently, β25-30 (0.3-30 µM) significantly reduced cell viability in both high and low density cultures. Combination of ACT (10 µg/ml) with β25-30 did not affect the toxicity of β25-30 in high and low density cultures (F-value = 2.22 and 0.61, respectively). These results suggested that both ACT and β25-30 were neurotoxic but that there was no synergistic interaction between them.

β-amyloid and heparan sulfate are found in neuritic senile plaques in Alzheimer's disease (AD). The co-localization of heparan sulfate and amyloidogenic proteins is a common feature of amyloidoses. β-amyloid 1-42 has been proposed to be toxic to neurons in vitro, and aggregation and toxic activities have been associated with amino acids 25-35. The role of heparan sulfate in the pathogenesis of amyloidoses however, is unknown. To investigate this interaction, we examined the effect of heparan sulfate on β-amyloid in a cell culture model, rat hippocampal neurons were exposed simultaneously to heparan sulfate and amyloidogen and either 75 μM β25-35 (a 25-35-mutant) or 25-35 (β25-35 induces degeneration and neuronal death in a dose-dependent manner with an optimal effective dose at 15μM. Concentrations as low as 3–5 μM were observed to have some protective influence. Heparan sulfate alone at these concentrations did not significantly increase cell survival. Heparan sulfate had no significant influence on β1-42 induced toxicity, even when observed at concentrations as high as 80 μg/mL. These data suggest that the neurotoxic effects of β25-35 may not be identical to those of the full-length peptide. It is further suggested that β1-42 retains its biological activity in its native state, however the activity of smaller fragments of β1-42 may be modified by heparan sulfate.

PATHOGENIC INTERACTIONS BETWEEN CIQ AND β-AMYLOID 8 PEPTIDE IN ALZHEIMER'S DISEASE. Scott Webster and Joseph Tsong. Sun Health Research Institute, Sun City, AZ 85373.

Soluble amyloid β-peptide (Aβ) is present in CSF of normal individuals. This (and others) has made it increasingly important to identify mechanisms by which Aβ becomes aggregated into biologically relevant, pathogenic fibrils. Aβ is present in the amyloidotic brain. We have previously shown that CIQ, the first component of the classical complement pathway, binds to synthetic Aβ peptides. Such binding activates the classical complement cascade and potentiates Aβ aggregation in vitro.

The present studies had two objectives. First, we modified a previously reported fluorometric assay so as to demonstrate dramatic enhancement of Aβ aggregation by CIQ at physiologically relevant, nanomolar concentrations. Second, we employed enzymatically generated CIQ fragments to characterize the CIQ binding site for Aβ. The latter appears to be within the globular head region (GHR) of CIQ, a finding that may clarify not only the mechanism by which CIQ augments Aβ aggregation and β structure, but also the mechanism by which CIQ activates CIQ. The GHR provides multiple binding sites for the Fc region of Ig. By analogy, it must also provide multiple binding sites for Aβ, an optimal quality for accelerating Aβ aggregation. Likewise, Aβ and Ig share a similar target for binding and activating CIQ, the GHR. Thus, complement activation in the AD brain is interactive with and may even be a consequence of Aβ aggregation into its pathogenic form.

Society for Neuroscience Abstracts, Volume 20, 1994


Neurodegenerative changes in Alzheimer's disease (AD) have been hypothesized to be mediated by β-amyloid protein (Aβ), an amphipathic peptide composed of a 42-residue fragment of the β-amyloid precursor protein. Previous studies have shown that the α-helical form of Aβ is toxic to cultured neurons, and that the Aβ25-35 fragment may be the active region mediating this toxicity. To determine if Aβ binds to neurons via a specific ligand-receptor interaction, we synthesized (D) and (L) stereoisomers of Aβ25-35. Purified peptides were shown to agitate on HPLC, Phase-contrast microscopy, electron microscopy and sedimentation analysis revealed that the two peptides exhibited nearly identical structural characteristics and aggregation levels. In addition, both isomers induce neurite loss in cultured hippocampal neurons. These data suggest that the neurotoxic actions of Aβ do not result from classic stereospecific ligand-receptor interactions. Rather, Aβ neurotoxicity may result from alterations in surface proteins, perturbation of cellular membranes, or other processes in which fibril features of the amyloidogenic peptide are important. This possibility is consistent with recent reports of similar neurotoxic actions by amylin and prion, amyloidogenic proteins which share structural similarities with Aβ but that have not been shown to mediate neurotoxicity. The mechanism of Aβ-induced toxicity will likely benefit attempts to understand the neurodegeneration that occurs in AD and perhaps other amyloid-related disorders.


Clusterin (apo J, SGP-2, ADHC-9, SP-40,40) was recently found bound to Aβ in cerebrospinal fluid and proposed to be a chaperone for Aβ [Ghisla et al. (1993) Biochem. J. 293:27]. Since the aggregation/conformational state of Aβ is important for its neurotoxicity, interactions with clusterin may affect Aβ toxicity. To test the hypothesis that clusterin may modulate Aβ neurotoxicity, we expressed human clusterin (apoJ, ADHC-9, 92717) in CHO-K1 cells and a C-terminal fragment of apoJ (Bt184, kbp) DNA into the expression vector pG-T and stably transfecting the hamster cell line AV-12. Clusterin was purified from conditioned media between 10-60% homogeneity by anion exchange and size exclusion chromatography. To study the putative relationship between clusterin and Aβ, we evaluated Aβ neurotoxicity in the presence and absence of clusterin using primary hippocampal cultures from fetal (E18) rats. Clusterin activity was assessed using 50 nM Aβ (Bachem lot ZK140), a well-characterized lot of Aβ that shows prominent neurotoxicity over a four-day timecourse. Co-treatment for four days with 50 μM Aβ and 31.25 nM to 500 μM clusterin, but not BSA or PBS controls, resulted in Aβ neuroprotection in a dose-dependent manner. These results suggest that clusterin may interfere with the adoption of a neurotoxic conformation/aggregation state of Aβ in vitro and may have a similar function in vivo.

AGGREGATED β-AMYLOID INDUCES REACTIVE ASTROCYTOYSIS BOTH IN VITRO AND IN ALZHEIMER'S BRAIN. C.I. Pike, R.L. Cummings, and C.W. Cotman. Irvine Research Unit in Brain Aging, University of California, Irvine, CA 92717 USA.

The senile plaques characteristic of Alzheimer's disease (AD) are often associated with astrocytes expressing a reactive phenotype. Since β-amyloid (Aβ) is the primary component of plaques, reactive astrocytosis in AD may result in part from direct effects of Aβ on astrocytes. To investigate this possibility, we first examined the effects of Aβ peptides on cultured astrocytes. Aβ did not induce significant cell loss in these cultures. However, upon exposure to aggregated but not soluble Aβ, astrocytes rapidly and stably adopted a stellate, process-bearing morphology consistent with the reactive phenotype. This altered morphology was accompanied by increased immunoreactivities for two markers of reactivity, basic fibroblast growth factor (BFGF) and glial fibroblast acidic protein (GFAP). In addition, similar to their interaction with senile plaques, astrocyte reactive processes in vitro were able to envelope Aβ aggregates. To investigate whether Aβ may induce similar effects on astrocytes in the AD brain, we triple-labeled sections of hippocampal formation from six AD subjects with thioflavine-S and antibodies raised against GFAP and either Aβ or BFGF. Upon scoring the intensity of each label within individual plaques, we found that reactive astrocytes were co-localized specifically with plaques expressing positive but not negative thioflavine staining, a marker of β-sheet structure characteristic of aggregated Aβ. Similar to the in vitro findings, we observed increased immunoreactivity for BFGF in reactive, plaque-associated astrocytes. These data suggest that Aβ, in an aggregation-dependent manner, can induce a reactive astrocyte phenotype. Thus, in addition to its hypothesized direct neurodegenerative effects, Aβ may indirectly affect the progression of AD via reactive astrocytosis.


The fibrilisation and cytotoxicity of various linear fragments of the amyloid β-peptide (25-35) fragment are being studied in order to determine the sequence and conformational requirements for these properties. Previous reports have demonstrated the necessity of the β-peptide (1-40) and the (25-35) fragment (Behl et al. 1992 BBRC 186, 544) a correlation between fibrilisation and cytotoxicity (Pike et al. 1993 J. Neurosci. 13, 1676) and also a cell surface binding requirement (JBD unpublished observation) but have not related them to sequence. The fibrilisation and cytotoxic properties of analogues of the amyloid β-peptide (25-35) fragment, containing single alanine or Glu substitutions were tested. Peptides which did not fibrillise were found to be non-toxic, confirming a requirement for peptide aggregation. Loss of activity might be attributed to either loss of non-specific peptide aggregation, (ii) loss of quaternary structure, (iii) an additional loss of sequence specificity, due to the substitution. Chemical aggregation of the peptide to BSA failed to rescue activity, suggesting that the tertiary structure of the aggregate is important for toxicity. Future substitutions will further define β-peptide's determinants for cell surface binding.

It has been suggested that the aggregation of amyloid B-protein enhances its neurotoxicity, and may play a key role in the Danielite of senile plaques. We revealed that aluminum, an epidemiologic risk factor for Alzheimer's disease, promoted the aggregation of synthetic amyloid B-protein (1-40) using immunoblotting and centrifugation (KAWAHARA et al., B.B.R.C., 198; 235:3-7 (1994)). Here, we report that significantly aggregated 1-40 has neurotoxic effects on cultured rat hippocampal neurons.

After incubation at 37°C for 24 h in 25 mM HEPES buffer (pH 7.0), 1-40 (obtained from BACHEM) was separated by SDS-PAGE and immunostained by antibody to amyloid B-protein (nl06); the in excess of 500 μM of n20, 1-40 formed aggregates from higher molecular weight up to the gel top. The aggregation was inhibited by the presence of detexoflene, a chelator of aluminum. Other metals including Zn2+, Co2+, Fe3+ caused only a small degree of aggregation compared to Al. Pre-treated solutions of 1-40 (aged), pre-treated solutions with Al (aged &Al), and 1-40 without pre-treatment (new-Al) were added to cultured hippocampal neurons obtained from 18-day embryo after 8-10 days in culture. After 7 days, cells were fixed and stained by MAB2 and SA3. Neurons treated with aged-Al showed a remarkable degeneration compared to aged or new-Al. The neurites of the neurons were also damaged. Widespread fibrillar deposits of 1-40 appeared on cells treated with aged-Al and aged-Al, but not with new-Al. More condensed and characteristic deposits were observed in aged-Al. These results suggested that the degeneration of neurons and the deposition of amyloid B-protein are enhanced by Al.

508.19


Aβ25-35 is known to induce neurodegeneration in cultures of rat brain cells and rat neural cell lines (Yamaguchi et al., 1990, Science, 250:279-282; Bohs et al., 1992, Biochem. Biophys. Res. Comm. 186: 944-950). The current data show that these peptides induce similar neurodegeneration in SH-SY5Y neuroblastoma cells, extending characterization of Aβ toxicity to a human nerve cell line. SH-SY5Y cells were aggregated with Aβ with changes in cell shape, membrane blebbing, antigenic modification, loss of attachment to the substrate, and cell death. Aβ25-35 induces aggregation for maximum toxic effects, as cellular degeneration is evoked by aggregated Aβ 1-42 and aggregated Aβ 41-41 (Ile to Cys) but not by monomeric Aβ 1-40. Aggregated (pre-aggregated) Aβ 1-42 also evoked neurodegeneration. 

Antigenic changes comprise upregulation of Alzheimer’s type tau epitopes, recognized by the PHF-1 and A30-50 monoclonals. These changes in tau support the connectivity between this in vitro model and mechanisms leading to neurodegeneration in Alzheimer’s disease. A significant feature of the SH-SY5Y response is that cells must be differentiated before they become sensitive to the degeneration evoked by Aβ. Signaling pathways leading to Aβ-evoked neurodegeneration thus are under experimental control, becoming complete only when proliferating cells withdraw from the cell cycle and develop a postmitotic phenotype.

508.20


Transforming growth factor β1 (TGF-β1) is found in plaques of patients with Alzheimer’s disease. The β2-amyloid peptide (BAP), the major component of these plaques, has been shown to be directly toxic to neurons and has thus been linked to the pathogenesis of Alzheimer’s disease. In the present study we determined whether TGF-β1 is able to modulate BAP neurotoxicity and the synthesis of its precursor proteins (APP).

Cultured rat hippocampal neurons were exposed to 0.1 - 10 μM of the BAP fragments 25-35 (BAPP25-35) for a period of 5-6 days. Exposure to 1-10 μM BAP led to a significant decrease in neuronal viability, which was maximal at 1 μM. Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling demonstrated that neuronal injury was accompanied by extensive DNA fragmentation, an indication of “apoptotic” cell death. A single addition of TGF-β1 (10 ng/ml) inhibited both BAPP25-35 neurotoxicity and reduced the extent of DNA fragmentation. Repeated treatments with TGF-β1 (4 x 10 ng/ml) completely prevented the death of the hippocampal neurons. In contrast to BAP, its precursor protein (APP) is thought to actually have neuroprotective properties. We next determined the effects of TGF-β1 on APP expression both in cultured rat hippocampal neurons and in secondary cultures of astrocytes. Treatment with TGF-β1 (0.1 - 10 ng/ml) led to an increased neuronal and glial production of APP, as determined by Western blotting and immunocytochemistry with a monoclonal antibody specific for APP.

We conclude that, in addition to its effect on Ca2+ homeostasis and β-2 expression, an increase in APP expression could underlie the protective effects of TGF-β1 against BAP neurotoxicity.

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DEGENERATIVE DISEASE: ALZHEIMER’S NEUROPATHOLOGY AND NEUROTRANSMITTERS—OTHER SYSTEMS

509.1

PHOSPHOINOSITIDE HYDROLYSIS IN HUMAN POSTMORTEM BRAIN MEMBRANES: ALTERATIONS IN ALZHEIMER’S DISEASE. A. Popovici*, L. Song, R. Powers and B. S. Eiken, Dept. of Psychiatry, Univ. of Alabama, Birmingham, AL 35294.

Membranes prepared from postmortem human prefrontal cortex hydrolized exogenous [3H]phosphatidylinositol PI) when (i) phospholipase C (PLC) was stimulated with Ca2+; (ii) GTPγS or NaF was added to stimulate G-proteins coupled to PLC, or (iii) calcium, ACPC, histamine, ATP, or serotonin plus GTPγS were added with GTPγS. The only response to ATP was influenced significantly by the duration of the postmortem interval (measured between 5 and 21 hr; n = 16). There was a decline in responses to several stimulators correlated with increased age (measured between 18 and 100 yr; r = 23). Utilization of antibodies to block responses indicated that Ca2+ and PI-β were the active subtypes mediating human membrane PI hydrolysis. ATP induced the greatest responses of the agonists tested and results indicate that it acts through P2-purnergic receptors. Comparisons of membranes from Alzheimer’s disease and age-matched controls indicated that Ca2+ and PL-β were the active subtypes mediating PI hydrolysis. ATP induced the greatest responses of the agonists tested and results indicate that ATP acts through P2-purnergic receptors. Comparisons of membranes from Alzheimer’s disease and age-matched controls indicated that Ca2+ and PL-β were the active subtypes mediating PI hydrolysis.

509.2

PERIPHERAL BENZODIAZEPINE RECEPTORS IN LYMPHOCYTES OF PATIENTS WITH ALZHEIMER’S DISEASE AND MULTI-INFARCT DEMENTIA. C. Ferrari*, A. Serranti*, G. Bianchi, R. Casavuotta, R. Riva, G. Bianchetti* and L. Franzola, Dept. of Neurology, University of Milan, Monza and (1) Alzheimer’s Unit, Sacro Cuore Institute, Brescia, Italy.

Peripheral benzodiazepine receptors (PBR) are modified in brain areas in animal models of stress and in Alzheimer’s disease (AD) patients. Since PBR present also in circulating lymphocytes, where appear to modulate immunologic functions, we presently investigated possible modifications of PBR in lymphocytes of patients with dementia, to investigate their role as putative peripheral markers of neurochemical alterations in degenerative disorders. 15 AD, 11 multi-infarct dementia (MID) patients (NINDS-ADRDA and DSM-IIIR criteria) and 10 age and sex matched controls were selected for peripheral blood analysis after a lymphocyte preparation. Frozen lymphocyte pellets were resuspended and incubated with various concentrations of [3H]PBR-11935, specific ligand of PBR, to analyze receptor density (Bmax) and affinity (Kd). PBR Bmax in all controls was similar to Bmax of young volunteers; a high variability of Bmax was observed in AD, although the mean was unchanged respect to controls. On the contrary, MID patients presented PBR densities significantly reduced respect to controls. No change of Kd was observed in any patient population. These findings may indicate a biochemical etiogenic in different AD patients and a possible role of PBR in neurochemical modifications observed in degenerative disorders.
500.3

AXELOTOXIC EFFECTS OF MCI-252, A NOVEL PSYCHOACTIVE COMPOUND, ON LATERAL HIPPOCAMPAL NEURONS IN RODENTS. J. Bouchi, T. Yusa, A. Tobe, M. Kakef and K.-I. Salto*. Pharmaceutical Labs. Lab. 1, Mitsubushi Kasei Corporation, 1000 Kamiyama-cho, Midori-ku, Yokohama 222, JAPAN. Clinical Research Center, MRC.

Effects of compound MCI-252, (4-[(2-fluorophenyl)-6-methyl-2-(1-piperazinyl)thieno[2,3-d]pyrimidine monohydrate], on memory and attention were examined. In water maze task in rats, MCI-252 (1-10 mg/kg, p.o.) ameliorated scopolamine-induced amnesia. Consecutive administration of MCI-252 (0.3 and 1 mg/kg, p.o.) reversed the passive avoidance failure in the basal forebrain-lesioned rats. MCI-252 at 1-10 or 10 and 30 mg/kg, p.o. reversed the attention deficits in mongolian gerbils or rats, whose cortical noradrenergic neurons were destroyed by 6,7-dichloro-1-naphthyl-2-bromobenzylamine or 6-hydroxydopamine, respectively. In the range of doses used, MCI-252 showed no abnormal behavior. Neurochemical studies showed that MCI-252 potentiated noradrenergic neuron pre- and post-synaptically, by inhibition of noradrenaline uptake ([C50+60%]) in vitro. Acetylcholinesterase inhibition in the DRN and the hippocampus was also observed.

500.4

STATUS OF SEROTONIN TRANSPORTER SITES (5-HTT) IN THE AGING HIPPOCAMPUS AND DORSAL RAPILE NUCLEUS (DRN) IN ALZHEIMER'S DISEASE. S. M. Tejani-Butt J. Yang J. Halak*, G. A. Ordway*, Dep't of Psychiatry, Univ. of Penn. School of Medicine, Philadelphia, PA 19104 and Dep't of Psychiatry & Human Behavior, Univ. of Mississippi Medical Center, Jackson, MS 39212.

There is increasing evidence that the functional integrity of the 5-HT system is compromised in age-related cognitive disorders. Several reports have indicated neurochemical changes in the DRN, including modest to severe losses of DRN neurons in Alzheimer's Disease (AD). Since ~40% of DRN neurons are considered to be 5-HT immunoreactive, this study analyzed 5-HT binding sites in order to obtain a quantitative assessment of the status of 5-HT innervation in the DRN in AD and the aging hippocampus. Sections were taken from the mid-region of the DRN from 8 subjects who had died of AD and 8 controls, and from the mid-region of the hippocampus from 18 subjects who had died of natural causes, ranging in ages from 19-80 years. A significant decrease in 3H-dextramipramine binding to 5-HTT sites was seen in the DRN in AD cases versus controls, with the most significant reductions (~30%) occurring in the lateral wings (p<0.01) of the DRN complex. In contrast, regression analysis of a significant effect of age on 5-HTT binding sites in the hippocampus (dentate gyrus, CA1, CA2, CA3) and entorhinal cortex. The results indicate that the integrity of 5-HT neurons in the DRN may be compromised, whereas the integrity of 5-HT terminals in the hippocampus may be unaffected by age. (Research funds from USDA grants NS 31699).

500.5


We have previously described the autoradiographic mapping of D2-like receptors with [3H]spiperone in human brain (Joyce et al., JPET, 255:1253, 1991), showing high expression in limbic regions. Reductions in the number of D2-like receptors in the DG, CA3, subiculum and perirhinal regions are observed in Alzheimer’s disease (AD) (Joyce et al., 1993; Neurosci. Lett. 190:33-38). In the present study we have observed a significant reduction in D2 receptor expression in the temporal cortex of Alzheimer’s patients, as compared to normal controls. The temporal cortices of AD patients showed a significant decrease in D2 receptor binding, as compared to normal controls. We also observed a significant reduction in the number of D2 receptors in the hippocampus of AD patients, as compared to normal controls. These findings suggest that the D2 receptor system may be a potential biomarker for the diagnosis of Alzheimer’s disease.

500.6

Differential regulation of RNA editing for an AMPA receptor subunit gene in the prefrontal cortex of Alzheimer’s patients: implications for schizophrenia and controls. S. Aebischer*, M.A. Smith and E.G. Jones. Dept of Anatomy and Neurobiology, University of California Irvine, CA 92717.

Studies in rat brain have demonstrated that the 5H4 cysteine to seryl mutation in the GluR-B subunit of the AMPA receptor is subject to RNA editing, by which a selected glutamine codon (CAG) is changed to an arginine codon (CGG). AMPA receptors containing GluR-B (2) polypeptide derived from edited RNA show a significant decrease in glutamate potentiation in comparison to AMPA receptors containing GluR-B (2) polypeptide derived from unedited RNA (Johnson et al., 1991, Cell 67:111). In the rat, the ratio of unedited vs. edited GluR-B (2) RNA changes from 1:99 during early brain development to 0:99:99 in the adult brain (Barnes et al., 1992, Neurosci. Lett. 139:195).

Dysregulation of GluR-B (2) editing could potentially cause AMPA and Cys2* mediated neurotoxicity. In the present study, the ratio and percentages of unedited and edited GluR-B (2) mRNA was analyzed in the prefrontal cortex of Alzheimer’s patients, schizophrenics and controls, using polymerase chain reaction with radiolabeled oligonucleotides, restriction enzyme analysis and scintillation radiography. In the Alzheimer’s brain, the abundance of unedited RNA was tenfold higher in comparison to age-matched controls (1.06% vs. 0.04%, p < 0.05) while in age-matched schizophrenics it was 0.39%. The abnormally high proportion of unedited GluR-B (2) mRNA in the Alzheimer’s brain indicates that RNA editing for GluR-B (2) subunit is compromised and could potentially be involved in mechanisms that underly chronic neurodegeneration. It will be of interest to study possible dysfunction of AMPA/KAR receptor RNA editing in the neurodegenerative diseases such as Amyotrophic lateral sclerosis or Huntington’s disease. Supported by NIMH grant MH44118 and by NARSAD and Stanley Grants.

500.7


Recently it has been reported that the cytosolic C-terminal amyloid precursor protein (APP) fragment, H165-Lys 676, forms a complex with isolated G<sub>a</sub>, a major GTP-binding subunit of APP (Goldsmith et al., 1991, Soc Neurosci. Abstr.). In this study we investigated the effect of the APP fragment H165-Lys 676 (APP 676), its reverse sequence and the smaller APP fragment Gly 659-Lys 676 on calcium currents in NG108-15 hybrid cells. In addition, we have investigated the interaction of these peptides with calcium current suppression mediated by adrenogenic a<sub>n</sub> and opioid a<sub>o</sub> receptors.

APP H165-Lys 676 (6x10<sup>-7</sup>M) did not affect calcium current suppression per se but clearly blocked calcium current suppression mediated by both adrenergic a<sub>n</sub> and opioid a<sub>o</sub> receptors in a concentration-dependent manner. The reverse APP fragment H165-Lys 676 and the shorter APP fragment Gly 659-Lys 676 did not affect calcium current suppression by adrenogenic a<sub>n</sub> and opioid a<sub>o</sub> receptors.

Our results show an interaction of C-terminal APP with adrenogenic a<sub>n</sub> and opioid a<sub>o</sub> receptor-mediated calcium current suppression downstream of the receptor, possibly via the GTP binding protein G<sub>a</sub>.Kishimoto I., Okamoto T., Matsuura T., Takahashi S., Okamoto T., Murayama Y., Ogita E., Nature, 362, 75-79, 1993.

500.8

REGULATION OF THE OLFACCTORY BULB ALZHEIMER’S PRECURSOR PROTEIN (APP) BY LESIONING OF THE OLFACCTORY BULB. M.C. Wilson, V. Ramakumar, R. Wang, B.N. Dharmi, B. Steinhoff, Dep’t of Pharmacology, Southern Illinois University School of Medicine, P.O. Box 19230, Springfield, IL 62794-9230 USA

Recent evidence suggests that APP modulates the activity of the guanine nucleotide regulatory protein, G<sub>a</sub>, a major growth cone protein. Growth cone associated protein (GAP-43) stimulates the binding of G<sub>a</sub> to GAP-43, thereby promoting its activation. Olfactory nerve deafferentation of the olfactory nerve led to an approximately 2-fold increase in GAP-43 to 4 weeks which is associated with regeneration of the olfactory nerve (Vergahein et al., J. Neurosci. Res. 26, 31-44). This study determined whether other components of this signalling pathway are altered by olfactory nerve lesioning. Lesioning was performed by administering a single injection of diethylthiocarbamate (DDTC) to Wistar rats (200-250 g). Vehicle-injected animals were used as controls. The levels of APP, GAP-43 and GAP-43 were determined by Western blotting. Nerve lesioning produced a transient increase in expression of APP (2-3 fold) by 6 weeks that recovered to control levels by 12 weeks. The levels of GAP-43 were also transiently increased (2-3 fold) by 6 weeks that recovered to control levels by 12 weeks. In contrast, GAP-43 level was decreased (~3 fold) over this time period and showed partial recovery (70-80% of control level) by 12 weeks. This upregulation of APP and GAP-43 might represent an attempt by the cell to compensate for the decrease in GAP-43. Olfactory nerve lesioning might provide a useful in vivo model to study.

We have previously shown that the β-amyloid precursor protein (β-APP) gene promoter is activated by the transcription factor c-Jun via a functional distal AP-1 site. We propose that stimuli that lead to activated Ras-dependent pathways would also regulate AP-1 activity to induce β-APP expression. Human astroglial cells (1321N1) were transiently co-transfected with a β-APP promoter luciferase reporter gene (a 593bp HindIII/ BamHI proximal promoter fragment fused to a human β-APP gene promoter) along with an expression vector for constitutively activated Ras. Our results show that expression of activated Ras increases β-APP-luc promoter activity approximately 5-fold; suggesting that stimulation of the Ras pathway can activate the β-APP promoter. Further experiments were carried out with a mutated form of the β-APP promoter containing a dysfunctional distal AP-1 site (Mut-β-APP-luc) which did not respond to co-expression of c-Jun or c-Fos. The Mut-β-APP-luc promoter was not inducible by activated Ras. We conclude that stimulation of the ras pathway leads to transactivation of β-APP expression via its distal AP-1 site. Studies are currently under way using a dominant negative Ras expression construct to establish a requirement for Ras in the regulation of the β-APP promoter activity by cellular mediators.


In comparison to control brain samples it has previously been shown that approximately 80% of Alzheimer’s Disease (AD) brains have the GTP binding site on β-tubulin substantially blocked and unable to be photoaffixed with 32P[GTPyS]. Also, addition of the cystolic fraction of AD brain homogenates was able to decrease this photoaffinity in control brains (Khaton et al., Ann. Neurol. 26, 210, 1989). Now we report that CSF from AD patients contains a very selective inhibitor of 32P[GTPyS] GTP co-precipitation into β-tubulin of control brain. This inhibitor is a flow through of centrifugation filtration and is stable to heat treatments of 90°C for 10 min. Treatment of CSF with calcium binding resins enhanced inhibitory activity whereas treatment with anion binding resins or with alkaline phosphatase totally abolished inhibitory activity. Ion exchange HPLC of cation filtered CSF and testing of the fractions for ability to reduce 32P[GTPyS] GTP co-precipitation identified one UV absorbing compound with an absorbance max of 291nm that blocked β-tubulin photoaffix. This compound was tentatively identified as a phosphorylated form of 8-hydroxyguanosine or 8-hydroxyguanosine. It is proposed that levels of this compound in the CSF may be a measure of the levels of hydroxyl radical (OH) or singlet oxygen (O2) in the brain. Also, these results imply that the radicals or endoperoxides formed by OH or O2 leading to 8-hydroxyguanosine nucleotides, may interact with various GTP binding proteins permanently modifying their biochemical properties. Supported by NIH grant GM 35766 and the Lincoln Clinic Foundation.


Increased release of excitatory amino acids (EAA) has been proposed to be involved in the pathogenesis of degenerative brain diseases, such as Alzheimer’s disease (AD). However, inconsistent changes in the cerebrospinal fluid (CSF) concentrations of EAA have been reported in patients with AD compared to controls, perhaps because the patients studied had illnesses of mixed severity. To determine whether there are stage-specific changes in CSF concentrations of EAA in AD patients with AD, we measured the CSF concentrations of glutamate, aspartate, and taurine (a comparison neutral amino acid) using high pressure liquid chromatography in 11 AD patients and 11 controls, recruited from the Alzheimer’s Disease Research Center. Twenty patients had early AD (CDR stage 0.5-1.0) and 12 patients had advanced AD (CDR stage 2.0-3.0). ANOVA revealed a significant increase in aspartate concentrations in advanced AD patients compared to controls (P<.15, P=.001, post-hoc Fisher’s PLSD - normals = early AD < advanced AD). A similar trend was observed for CSF glutamate concentrations (P=.24, P=.095). No significant differences in CSF taurine concentrations were observed among the groups. CSF aspartate concentrations were highly correlated with serum aspartate concentrations (r=.84, P=.0001), but not with taurine concentrations. These results indicate that CSF EAA concentrations are abnormally increased in patients with advanced but not early AD, and suggest that increased EAA levels may play a pathophysiological role late in the disease. This project was supported by the Alzheimer’s Disease Research Center Grant P50 AG05681.


Impaired energy metabolism can induce excitotoxic neuronal death and may contribute to the pathogenesis of late-onset neurodegenerative disorders such as Alzheimer’s disease. To determine if impaired energy metabolism could play a causal role in the formation of neurofibrillary tangles, we administered the mitochondrial inhibitor malonate (a reversible inhibitor of succinate dehydrogenase) to rats via intrahippocampal injection. Immunocytochemistry was used to examine cytoskeletal changes at different post-injection time points. Within six hours after injection, malonate (2 μmol) resulted in neuronal cell death, loss of MAP2 and MAP1B, and relative sparing of tau in the hippocampus. At the drug injection site, damage was widespread and involved most cell types in the hippocampal formation. Dilation to the injection site CA1 neurons were most vulnerable, although CA3 neurons were also affected. Intrahippocampal injection of the excitotoxin quinolinate resulted in a similar pattern of cell loss and cytoskeletal disruption. These results support the hypothesis that metabolic inhibition and excitotoxic insult may contribute to the sparing of tau and loss of other cytoskeletal proteins in tangle-bearing neurons in Alzheimer’s disease. Supported by AG10678.


In a previous study (Brain Research 726, 2004) we employed immunohistochemical techniques to demonstrate a decrease in GluR2/3 and GluR1 immunoreactivity in the entorhinal cortex (EC) of patients with Alzheimer’s disease. In the current study, Western blot analysis is employed in order to determine the extent to which selected GluR subunits are reduced in the EC. Moreover, we examined whether the decrease in GluR2/3 immunoreactivity within layer II pyramidal neurons in the EC parallels alterations in the expression of various cytoskeletal markers. Throughout these studies we examined normal subject controls and compared varying degrees of AD pathology. In patients with clinical and pathological verification of AD, GluR2/3 protein was reduced by nearly 50% of controls while GluR1 was decreased by 40%. Cell counts of GluR2/3 immunoreactive neurons demonstrated that the reduction of GluR2/3 could be attributed largely to a loss of layer IIb fibrous cells. In contrast, labeled cells were abundant in layers V and VI even in those cases with severe AD pathology. Examination of non-entorhinal cortical areas revealed a decrease in GluR2/3 protein, but no GluR1 immunoreactive neurons. The decline in GluR2/3 protein in the EC is accompanied by a decrease in synaptic contacts contributing to cytoskeletal pathology. This work is supported by NIH grant AG04206 and The American Health Assistance Foundation.
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DEGENERATIVE DISEASE: ALZHEIMER'S—CELLULAR MECHANISMS OF DEGENERATION


The entorhinal cortex provides a major input to the ipsilateral hippocampal dentate gyrus. A unilateral entorhinal cortex lesion preserves the outer molecular layer triggering synapticosis and the replacement of synaptic connections. This reactive replacement of synaptic connections was originally studied with quantitative electron microscopy (EM). More recent experiments have employed synaptophysin-immunoreactivity (SI) to quantitatively assess synapse numbers utilizing light microscopy. No one has directly compared actual numbers of synapses observed with EM to values obtained with SI. Adult Fisher 344 rats were subjected to a unilateral entorhinal cortex lesion and killed at 2, 4, 10, 15, 30 and 60 days after the injury. Tissue was processed for both immunoreactivity and utilizing an antibody directed against synaptophysin, and for EM employing standard techniques. For the EM prepared tissue, the number of synapses per 100 µm² was ascertained. An image analysis device was used to obtain the corrected optical density for the SI prepared tissue.

Both techniques demonstrated a loss of synaptic contacts immediately following the lesion, although the magnitude was significantly less for the SI material. A time dependent change in staining could be observed in the SI material which, mimicked but did not parallel the rate and magnitude of synapse replacement observed with EM. The present results call into question the use of SI for the accurate quantitative assessment of synaptic number. Supported by AG05144.

510.2 SYNAPTIC DENSITY IN THE HIPPOCAMPAL DENTATE GYRUS IN ALZHEIMER PATIENTS WITH ALZHEIMER'S DISEASE. D.A. Price, D.L. Sparks* and S.W. Scheff Center On Aging, University of Kentucky, Lexington, KY 40536-0370.

Substantial synapse loss occurs in many cortical areas associated with Alzheimer's disease (AD). These brain regions are primary association cortex which normally receive significant inputs from many other association regions known to have AD pathology. The hippocampal formation is one of the primary areas affected in AD, and responsible in part for some of the impaired memory and dementia characteristics of the disease. We previously reported a substantial loss of synaptic numbers in the outer molecular layer of the hippocampal dentate gyrus. This area normally receives a direct input from the ipsilateral entorhinal cortex which has significant neuronal loss in AD. The inner molecular layer of the dentate gyrus receives most of its input from within the ipsilateral and contralateral hippocampi, and may not be affected by the disease process. The present study determined whether or not this area of the hippocampus is capable of maintaining synaptic numbers in AD patients as compared to neurologically normal controls.

Human brains were obtained from postmortem examination from patients who met the NINCDS-ADRSA criteria for AD and from age-matched controls. All tissues were obtained within 10 hours postmortem. The tissue was coded and prepared for ultrastructural examination. The density of synapses and the synaptic apposition length was determined for each subject. Because of ongoing research with these tissues the group designation remains coded at this time but will be discussed in full at the meetings. Correlations with synaptic density in the outer molecular layer will be presented. Supported by AG05144.

510.3 SYNAPTIC ANTIGEN REACTIVITY WITH A SERIES OF MONOCLONAL ANTIBODIES. A. Prusiner*, R. Bowser, W.O. Honig* and P. Davies. Dept. of Pathology, Albert Einstein College of Medicine, Bronx, NY and (1) Dept. of Psychiatry, Univ. of British Columbia, Vancouver, BC (Canada).

In a previous report, a series of 16 monoclonal antibodies (the SP series) to presumed synaptic antigens were produced. (W.G. et al., Brain Research 609:9-20 (1993)). This report is to verify that these antibodies do, in fact, react with synaptic antigens. The antibodies were produced by injecting mice with protein from whole brain. For one group of antibodies which was partially purified by eluting from an affinity column of the monoclonal antibody, which reacts with a synaptophysin-like antigen, attached to a peptide. Antigens presumably associated with synaptophysin in synaptic vesicles or membrane fractions would also be enriched by this method. Normal brain homogenates were prepared and fractionated to produce a synaptic vesicle rich fraction. Electrophoresis on a 12% acrylamide gel was performed and immunoblotting was done with each of the 16 SP series antibodies, along with EP10 and a commercial anti-synaptophysin antibody. When equal amounts of protein from each of the homogenates were analyzed, there was a pattern of enrichment of the antigens in the synaptic vesicle fraction. These antibodies, therefore, are reacting with antigens associated with the synaptic vesicle fraction, as predicted. We have confirmed that the SP monoclonals are reactive with five different proteins of this fraction. Further work with synaptic vesicle fractions isolated from Alzheimer's disease brains is ongoing.


Fibrillary tangles and amyloid plaques, the traditional markers of Alzheimer's disease (AD), are identified only post-mortem, and are present to a varying degree in nondemented aged brain as well. In addition, a definitive causative role in AD, for either of these complexes and their associated protein components, has not yet been shown. Here, we have attempted to identify proteins in cortex of AD brain as potential markers of the disease using high resolution two-dimensional electrophoresis in an effort to provide immune-reactive targets more specific for AD and perhaps more indicative of AD etiology. Protein from prefrontal cortex of AD and age-matched control brain was extracted, solubilized and separated first by isoelectric point, followed by molecular weight using the Millipore 2-DE electrophoretic system. The fractionation between 23 and 70 kDa and pH 3.5-10 6-8 contained at least one or more spots as identified by silver and coomassie stain in AD samples that were not present in controls. One of these spots (20 KD) was targeted for further analysis by electrophorically blotting onto PVDF membrane. The target spot was cut out of the membrane after being revealed by coomassie stain and sequenced using an automated amino acid sequencer. Two sequences were obtained and used to identify a unique peptide sequence, both beginning five residues from the N-terminus. These peptides were identical, except for a single amino acid residue (IaW) at position nine in the peptide or position 14 from the N-terminus of the protein. These peptides were injected into rabbits and antisera for each peptide was recovered and used to identify the target spot. However, immunobots using the A peptide revealed three or more spots in control and five or more spots in AD samples within the same gel region but with a much larger molecular weight and possibly greater abundance than the target spot. Furthermore, the A to W amino acid substitution completely changed the specificity of the antibody, revealing a single spot within the same molecular weight region detectable in AD as well as normal brain. Results obtained in this study suggest, assuming the accessibility of antibody to the antigenic site of the proteins, the possibility of an alternative processing of proteins observed in AD relative to controls.

WEDNESDAY PM

510.5 NEUROTRANSMITTERS—OTHER SYSTEMS

DEGENERATIVE DISEASE: ALZHEIMER'S: NEUROPATHOLOGY AND NEUROTRANSmitters—OTHER SYSTEMS

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510.5 CHARACTERIZATION OF apl-1, AN AMYLID PRECURSOR-LIKE GENE IN THE NEMATODE CAENORHABDIS ELEGANS. I. Dangle*, C. Li, and D. Li, Dept. of Biology, Boston University, Boston, MA 02215

Alzheimer's disease is a neurodegenerative disease for which the cause is still unknown. Accumulation of a β-amyloid peptide in the brain of patients has been associated with the disease. This β peptide is derived from a larger amyloid protein precursor (APP), for which the normal function and processing remain to be understood. Recently, a family of APP-related proteins have been identified in Caenorhabditis elegans. We are interested in elucidating the function of APP by looking at APP-related genes in the nematode Caenorhabditis elegans.

We have previously reported the isolation and characterization of an APP-related cDNA from C. elegans, apl-1, and have shown that the predicted APL-1 protein is a member of the APP family of proteins. The genomic region corresponding to apl-1 has been identified and characterized; apl-1 spans 4 kb and contains 12 exons and 11 introns. It has been positioned to the left arm of the X chromosome of C. elegans. We are interested in studying the function of apl-1 by generating "knockout" worms, using a transposon insertional inactivation strategy. A mutant strain containing a transposon inserted in apl-1 has been isolated by Ron Plasterk and kindly provided to us. The transposon insertion has been located to exon 12, prior to the transmembrane domain. Further characterization of the mutant phenotype is underway. To determine the expression pattern of APL-1, antibodies against different parts of APL-1 are being generated and will be used to perform immunocytochemistry.

510.7 CHARACTERISTICS OF SECRETASE CLEAVAGE OF APP FROM PC12 CELL MEMBRANES. J.A. Ripolles*, Y. Chen, S.B. Roberts, K.M. Fiehnlein*, and N.K. Robakis, Dept. of Psychiatry and Fishberg Center for Neuroscience, Mount Sinai Medical Center, New York, NY 10029, and Bristol-Myers Squibb, Wallingford, CT 06492

The primary component of amyloid plaques, the hallmark of pathology in Alzheimer's Disease, is a 39-43 amino acid long peptide known as the amyloid-β peptide. β-peptide is derived by proteolytic processing of a larger precursor protein known as Amyloid Precursor Protein (APP). One pathway for APP processing which leaves the β-peptide domain prevents the production of amyloidogenic products and occurs via an enzyme known as "secretase". In this report we show that a subcellular fraction of PC12 cell cultures is enriched in "secretase" activity. After incubation at 37°C of membranes devoid of peripherally-associated molecules, a soluble 120DK fragment of APP is produced which on 7% SDS-PAGE has the same mobility as purified, secreted APP. This APP species is recognized by N-terminal specific antibodies but is not recognized by C-terminal specific antibodies. The enzymatic activity resulting in the release of the Nifin II form of APP has a pH range of 7.5 to 10.0, is differentially regulated by divalent cations, and is inhibited by two serine protease inhibitors. Moreover, the activity appears to be tightly membrane associated since extraction with 0.5M NaCl or sodium carbonate did not reduce the production of Nifin II.


The cellular prion protein (PrP) is a 250 amino acid glycoprotein of unknown function, highly expressed in neurons. In transmissible and genetic neurodegenerative diseases such as scrapie of the sheep and Creutzfeldt-Jakob encephalopathy of the humans, PrP is converted in an altered form (PrPSc), identical in its primary structure but distinguishable from the normal protein for its resistance to protease digestion. In fact PrPSc accumulates in the brain of affected individuals in protease-resistant, amyloid-like aggregates. Recently, it was reported that a fragment of PrP (Pp106-126) was able to induce neuronal apoptotic death in vitro, and an APP peptide homolog was identified in amyloid fibrils. Since all the PrP-related neurodegenerative diseases are characterized by both neuronal death and astroglia, our study was aimed to identify a direct proliferative effect of primary cultures of cortical astrocytes of the PrP106-126 fragment and whether the cellular mechanisms were involved. Pp106-126 induced a remarkable increase in quiesce (growth in absence of serum) rat cortical astrocytes assayed at both initiated thymidine incorporation and excomaxidine proliferation assay. Specific antagonists for different intracellular pathways were used to identify the molecular mechanism of action of Pp106-126. (MURST 40% 1992, 1993 to G.S.)

510.9 EFFECTS OF ETHANOLAMINE (Etn) ADMINISTRATION ON THE LEVELS OF Etn AND CHOLINE IN PLASMA, BRAIN EXTRACELLULAR FLUID (ECF) AND BRAIN TISSUE, AND ON THE LEVELS OF BRAIN PHOSPHOLIPIDS IN RATS: AN IN VIVO AND IN VITRO STUDY. E. De Michelis and R. E. Wurtman Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139

The sources and fates of brain ethanolamine (Etn) are poorly known and the effects of its administration have not been investigated, even though the cortical levels of this base are shown to be reduced in neurodegenerative pathologies with hypocholinergic function like Alzheimer's disease and Huntington's disease. We studied the effect of i.p. different Etn doses (10-2, 10-3 and 10-4 M/kg) and choline's (Ch) levels in arterial plasma and brain extracellular fluid (ECF) of awake rats. We also studied its effects on brain levels of Etn, Ch, and their respective major phospholipids. Etn administration caused dose dependent increase in Etn levels within plasma (p<0.05) and brain ECF (p<0.05), and also in brain Etn (p<0.05) and phosphatidylcholine (p<0.05). Systemic Etn also increased Ch levels in plasma (not completely dose dependently), brain ECF (dose dependently) (p<0.05), and in brain tissue (p<0.05); brain levels of phosphatidylcholine also increased. Possible metabolic pathways relating these increased Etn are discussed, as well as possible mechanisms of the decrease brain Etn in Alzheimer's disease.

510.10 DISTRIBUTION AND SEMI-QUANTITATIVE ANALYSIS OF TYROSINE HYDROXYLASE mRNA IN THE NORMAL HUMAN AND ALZHEIMER'S DISEASE LOCUS CERULEUS J. Leven*, M.A. Miller, P.E. Klotz, E.R. Parkind, and M.A. Barkind, Departments of Medicine (Neurology) and Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195

Locus ceruleus neuronal loss is well documented in Alzheimer's disease, though concomitant decline of noradrenergic function has not been convincingly demonstrated. We have, therefore, become interested in the regulation and function of locus ceruleus neurons in both the normal state and in Alzheimer's disease. Brainstem with the complete locus ceruleus from both normal elderly patients and in Alzheimer's disease were collected and snap frozen. The entire locus ceruleus was serially sectioned from rostral to caudal extent and prepared for anatomical, immunohistochemical and in situ hybridization studies. We have developed in situ techniques for use in human brain tissue to examine for the presence and level of expression of tyrosine hydroxylase message using a 153 bp riboprobe (cDNA generously provided by Karen O'Malley, Washington Univ.). Our initial studies have demonstrated message for tyrosine hydroxylase in the locus ceruleus in both the normal and Alzheimer's disease brain. The findings are consistent with previous i.c.v. and i.v. tyrosine and rat brain. We will describe the distribution and level of expression of tyrosine hydroxylase message. We believe these studies will help elucidate the influence of Alzheimer's disease on locus ceruleus function and explain some of the paradoxes seen in the noradrenergic system in this disease.
LOCALIZATION OF THE DEFICIT IN CYTOCHROME OXIDASE (COX) ACTIVITY AND COX SUBUNIT mRNA WITHIN THE CEREBRAL CORTEX IN ALZHEIMER'S DISEASE (AD), K. Hastie, D.R. Brady, J. Shilt, S.J. Rapoport, C. Chandrasekaran, Laboratory of Neurosciences, NIA, Bethesda, MD 20205.

Quantitative COX histochemistry was used to measure COX activity in tissue sections from motor and temporal cortices of 12 AD and 7 control brains. The method was calibrated with tissue sections from homogenates of known COX activity. We also used in situ hybridization to localize mRNA of COX subunit III (COX III) and 16S rRNA, a marker of total mitochondrial RNA levels. To ensure identical hybridization conditions, slides containing sections from the temporal and motor cortex of the same patient were paired and hybridized with the sections facing each other, with the probe between them. Within the AD brain, COX activity is temporally cortex, a region typically affected in AD, was 29% lower in motor cortex (p<0.01), a region typically spared in AD. The levels of COX III mRNA in temporal cortex relative to motor cortex were 35% lower (p<0.01), while 16S rRNA showed no difference. Control cases showed no differences between the two brain regions in COX activity or COX III mRNA levels. There were no significant correlations between the decrease in COX III activity and the numbers of neurofibrillary tangles or senile plaques in the AD brains.

The levels of COX activity and mRNA were plotted as a function of distance from the pial surface. AD cases show a more pronounced decrease in layers I-II. Studies are under way to relate these deficits to the pre mortem clinical state of the disease, to loss of neurons and synapses, and to altered regulation of mitochondrial gene expression.

ACCUMULATION OF UBQUITIN-PROTEIN CONJUGATES IN A NEURONAL CELL LINE FOLLOWING TREATMENT WITH AN INHIBITOR OF THE MULTICATALYTIC PROTEINASE COMPLEX (250 PROTEASOME), M.E. Figueroa-Pereira, K.A. Bernt & S. Wilk, Deps of Pharmacology and Anesthesiology, Mount Sinai School of Medicine, CUNY, New York, New York 10029, USA.

Accumulation of ubiquitin-protein conjugates is common in abnormally inclusion inclusions of patients with idiopathic chronic neurodegenerative diseases. Covalent binding of ubiquitin to proteins is viewed as a means by which proteins are marked for subsequent degradation. We report that exposure of HT4 cells (a mouse neuronal cell line) to a potent inhibitor of the multicatalytic proteinase complex (MPC) causes accumulation of ubiquitinylated proteins. In contrast, incubations with a calpain inhibitor or with a lysosomal agent failed to produce detectable ubiquitinylated proteins. These results suggest that a malfunction in the ubiquitin/ATP-dependent protein degrading system, of which MPC is the "catalytic core", could be a factor in the accumulation of ubiquitin protein conjugates identified in neurodegenerative brains. The MPC-inhibitor may therefore be a useful tool for developing a model for the study of neurodegenerative diseases. (Supported by NIH grants NS-29036, MH-00350, GM-34582 and MH-48125.)

AN INFORMATIVE MICROSATellite [GT] REPEAT ON A CHROMOSOME 14 COSMID ALSO CONTAINING E2K, Ghazala Ali, Wilam Wesolowski, Rudolph Tanzi, and John Blass, "Burke Medical Research Institute, Cornell Medical College, White Plains, NY 10605 and Laboratory of Genetics & Aging, Massachusetts General Hospital, Charlestown, MA 02129.

Previous studies have indicated that the activity of the α-ketoglutarate dehydrogenase complex (E2K) is reduced in Alzheimer (AD) brain (Gibson et al, Arch Neurol 45:836-1988; Magistrocinco et al, J Neurochem 61:2007,1994) and fibroblasts (Cooper et al, Ann Neurol 35:519-1994). KDHIC consists of 3 proteins, E1K, E2K, and E3. We have localized E1K to chromosome 7 (Szabo et al, in press) and E2K to chromosome 14q243 (Ali et al, in press); E3 had previously been linked to chromosome 7. In order to test for association of the E2K gene with Alzheimer's disease, we have identified a microsatellite repeat region on a chromosome 14 cosmid which also binds the full-length E2K probe which we have isolated and sequenced recently (Tanzi et al, submitted).

Ten cosmids derived from a human chromosome 14 library were tested for cohybridization with E2K and with a GT repeat 30-mer. The one cosmids which was found to bind both probes was subcloned and sequenced. The 286 bp microsatellite region was used to design PCR primers amplifying a 401 bp fragment. This is an informative polymorphism giving rise to at least 10 separate common alleles. The polymorphism is being tested for association with familial and apparently sporadic AD.


**S10.17**

**APOPTOSIS IS INDUCED BY HYDROGEN PEROXIDE IN CULTURED CENTRAL NERVOUS SYSTEM NEURONS.** E.R. Whittemore, D.T. Lee, and C.W. Cooper, Brain Aging Unit, Irvine, CA 92717.

The accumulation of α-amyloid peptides (Aβ) in senile plaques is a principal event in the neuropathology of Alzheimer disease. We previously reported that CNS neurons undergo apoptosis following exposure to Aβ in vitro. A link between Aβ-mediated apoptosis and oxidative damage has been suggested by studies reporting that antioxidant agents protect neurons from Aβ-induced cell death. We have pursued this possibility and demonstrated that exposure of neurons to 100 μM hydrogen peroxide for 24 h induced apoptosis as assessed by toxicity assays, nuclear staining, and assessment of internucleosomal DNA fragmentation.

We have begun to examine in more detail the mechanism of induction of apoptosis in neurons by hydrogen peroxide. We report that a reduction in cell viability and mitochondrial function occurs within 3 h following hydrogen peroxide exposure, and parallels the induction of DNA fragmentation. In other models of apoptosis, increases in [Ca2+]i have been linked with the activation of endonucleases responsible for DNA fragmentation. Measurement of [Ca2+]i using fura-2 imaging following exposure of neurons to hydrogen peroxide indicates that the [Ca2+]i increases steadily over time, consistent with the idea of activation of a Ca2+-dependent endonuclease. Vulnerability to hydrogen peroxide is cell type specific within the CNS, with neurons being most sensitive, followed by microglia, then astrocytes. These results suggest that direct oxidative injury may serve as a general trigger for neuronal apoptosis in the CNS, and that apoptotic cell death may be induced by oxidative injury and/or stress that accompanies many neurodegenerative diseases.

**S10.18**


Our cytochemical studies demonstrate progressive disturbances of endosomal-lysosomal function in Alzheimer Disease (AD) pathogenesis. Lysosomal alterations were identified in normal-appearing, at-risk AD neurons and progress with advanced degeneration. Following cell lysis, lysosomal hydrolases are present extracellularly in amyloid-containing senile plaques (SP). To establish whether cytochemical alterations could be explained by changes at the molecular level, we examined the gene expression of cathepsin D (CD) in fetal frozen, AD and control brains (PMI ≤ 6.5 h). Serial sections of the prefrontal cortex were analyzed with CD antisem and by in situ hybridization (ISH). Changes in the levels of CD expression were evaluated by semiquantitative densitometric and Northern blot analyses. SP were identified by silver stain and thioflavin S. In AD brains, ≈ 75% of sequested perikarya in layers III and V displayed 2-fold higher levels of CD mRNA by ISH compared to control brains (p < 0.001). The maps showed neuronal populations contained increased CD immunoactivity. Elevated levels of CD mRNA were confirmed by Northern blot. CD mRNA signal was localized in the soma and dendrites, but not in SP immunoreactive with CD antisem. Our results indicate that lysosomal system alterations occur early in AD pathogenesis. Activation of the lysosomal system is manifested by increased CD gene expression and immunoreactivity in at-risk AD neurons. Changes in hydrolase expression may be one of the earliest indicators of selective cell vulnerability in certain AD neurons preceding other histological and cytochemical evidence of degeneration and may relate to the formation of amyloid-containing SP.

**S10.19**

**MEMBRANOUS LIPODYSTROPHY WITH ALZHEIMER’S SENILE PATHOLOGY.** Y. Takamura, R. Fukatsu, K. Tsumuki, Y. Yokoi, H. Takahashi, N. Itoh, N. Fuji, I. Kusunoki, T. Koyama, K. Naganuma, and N. Takahata. Dept. of Psychiatry and Pathology, Hokkaido Univ., Deps. of Neuropsychiatry and Microbiology Sapporo Medical Univ., 7Nakamura Memorial Hospital, Sapporo 060, Japan.

Membranous lipodystrophy (ML) is a rare disease characterized by multiple bone lesions with repetitive fractures and progressive neurosychiatric deterioration. We had an opportunity to examine two cases of the disease with Alzheimer’s senile change. In present study, we compare the senile changes in ML with those in Alzheimer’s disease.

**case 1.** 50 y old female. Brain weight 740 g. Severe generalized atrophy diffuse and profound demyelination and glisosis, sudanophilic granules, axonal swelling and spheroids were present. Wide spread senile plaques (SPs)and neurofibrillary tangles (NFTs) in the cortex.

**case 2.** 53 y old female. Brain weight 1030 g. Marked cerebral atrophy, severe demyelination and gliosis, numerous SPs and NFTs were found. SPs were distributed in cerebral cortices, predominantly in parietal and occipital lobes. The classical or compact plaques were predominant, but diffuse or subpial plaques were rare. Amyloid angiopathy were not recognized SPs were stained with anti β antibodies, and anti amyloid associated proteins Western blot analysis of brain extracts showed similar results.

In conclusion, the differences in senile alteration suggest that there are differences in pathogenesis of these senile changes between two cases extremely unusual cases and Alzheimer’s disease.

**S11.1**


Monkeys (Macaca fascicularis) with biotin acid (IA) lesions of the head of the caudate nucleus are characterized by anterograde and retrograde memory defects, and in vivo evidence of atrophy of the injected nucleus and ventricular enlargement on MRI. As in Huntington’s disease, overt signal change in regions of presumptive hypoelectricity is elusive on visual inspection. In this study, we mapped the distribution of signal intensity on proton density images (TR = 2000-2500, T1f = 42; in plane resolution = 0.47 mm).

The region containing the caudate nucleus was located by identifying pixel intensity transmissibility at the border of the caudate in the lateral ventricle, corpus callosum, corona radiata and internal capsule. Dorsal-ventral columns of pixels were averaged after subtracting background-adjacent pixels to reduce the weighting of the means by heavily myelinated areas, CSF and edema enhancing artifacts. In the normal caudate, signal intensity rose moderately from lateral to medial. In contrast, a local zone of hypointensity caused each IA injected caudate to depart from the linearity of the normal distribution. Regional deviations were approximately in register with the planned coordinates of the lesions. Loss of signal intensity may reflect neuronal depletion and gliosis, and thus the distribution of signal intensity may serve to be sensitive to the local anatomic and neurochemical consequences of neurotoxic tissue injury. However, hemosiderin deposits associated with microhemorrhage may also contribute. The findings may have relevance in assessing early state neuropathology in HD with structural imaging.

**S11.2**

**3-NITROPROPICIC ACID NEUROTOXICITY IN STRIATAL AND NON-STRIATAL CULTURES IS MODULATED BY ENERGY SUBSTRATE AVAILABILITY BUT NOT SIGNIFICANTLY BY GLUTAMATE RECEPTOR ANTAGONISTS.** S.L. Fink, D.Y. Ho*, and R.M. Sapolsky. Deps. of Neurosciences and Biological Sciences, Stanford Univ., Stanford, CA 94305-520.

3-Nitropiproic acid irreversibly inhibits the activity of the mitochondrial enzyme succinate dehydrogenase. When administered systemically to rats or ingested accidentally by humans or livestock, it causes striatal lesions. All reports thus far on 3-NP neuronal effects have used whole animals or cortical explants. We studied the effects of 3-NP on dissociated cultures of neurons and glia. We found that, despite its selective toxicity in vivo, intoxication with 3-NP leads to comparable dose-dependent neuron death in cultured striatal, hippocampal, and hypothalamic neurons. In addition, 3-NPs effects were remarkable energy substrate-dependent, with its toxicity being potentiated by low glucose conditions to a greater extent than was the case for glutamate. Furthermore, death of neuronal explants was exacerbated with a herpes simplex virus vesi, carrying GLUT-1 glucose transporter significantly protected them against toxicity vrex by modestly damaging doses of 3-NP in low glucose media. Finally, the striatal damage caused by 3-NP administration has been shown to be partially mediated by excitotoxins. This is supported by one report in which glutamate receptor antagonists attenuated 3-NP-induced damage in cortical explants (A. C. Lodoloh, et al, Neurodenger J, 155-161 (19923)). We found that inoculation with the non-specific glutamate receptor antagonist, kynurenate, increased neuronal survival from 15% to 61% of control in low glucose conditions, but did not significantly increase survival of neurons exposed to 3-NP. We are currently studying whether 3-NP toxicity can be decreased by co-inoculation of the cultures with kynurenate and DL-2-amino-7- phosphonopentanoic acid (APH), an NMDA-selective glutamate receptor antagonist. If so, this would support a role for excitotoxic-induced cellular injury in the neuronal damage by this enetic toxin; however, pilot experiments indicate that most of the toxicity is caused by other cellular processes.

**DEGENERATIVE DISEASE: OTHER—NEUROTOXIC EFFECTS AND HUNTINGTON’S**

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**S1245**

**SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994**
DEGENERATIVE DISEASE: OTHER—NEUROTOXIC EFFECTS AND HUNTINGTON’S

511.3 NEURONAL DEGENERATION INDUCED BY 3-NITROPYRUVIC ACID IN THE FOREBRAIN ANALYZE WITH A SILVER STAINING METHOD: FL. MIKULA AND L. ZABORSZYK. Center for Molecular & Behavioral Neuroscience, Rutgers, The State University of NJ, New Jersey, NJ 07102

3-Nitropyruric acid (3-NP) is a mitochondrial toxin which has been suggested (Beal et al., 1993) to replicate many of the histological and neurochemical characteristics of Huntington’s disease upon systemic or local striatal administration. The apparent neuronal loss in the forebrain is less than well understood. 3-NP was injected stereotaxically (500muM, 0.1-0.5uL) into various forebrain areas (striatum, internal capsule, thalamus, lateral hypothalamus, and hippocampus) in naive rats. While injections of 3-NP into the striatum resulted in local neuronal degeneration, an equally large number of medium-sized spiny striatal neurons degenerated if the injections were targeted. Injections of 3-NP also resulted in topographically organized neuronal degeneration in various thalamic nuclei, corresponding to the damage of their axons and/or terminals. Thalamic nuclei were always heavily affected. Moderate neuronal degeneration was also observed in the pars compacta and in some cases in the par reticulata of the substantia nigra. In spite of the fact that cortical axons were affected by the toxin, little cell death was observed in the cortex. These studies suggest that intracerebral injection of 3-NP can cause substantial retrograde degeneration in basal ganglia circuits, with different vulnerability patterns. This different vulnerability pattern might be taken into consideration when using 3-NP in an animal model of Huntington’s disease.

Supported by USPHS grants NS29545, NS30024.

511.5 HISTOLOGICAL AND BEHAVIORAL EFFECTS OF CHRONIC 3-NITROPYRUVIC ACID TREATMENT ON NASAL DOPAMINE RESPONSIVE REGIONS. GE Rousset1*, M-C Gayet-R Dolan, M. Mazibre and P. Hantraye. Service Hospitalier Frederic Joliot, CNRS URA125, 4 place du General Leclerc, 91401Codes Orsay, FRANCE.

Chronic administration of 3-nitropropionic acid (3-NP) in rats has been shown recently to produce bilateral forebrain lesions, closely replicating the neuropathology of Huntington’s disease (HD) (Beal et al., 1993). J. Neurosci. 13:4818-92. In the present study, we investigated the behavioral and anatomical effects of a chronic 3NP treatment (1mg/kg/day) on the performance of naive rats. Four months after the post-injection, 60-70% of 3NP-treated animals showed mild motor symptoms including a wobbling and an apparent decrease in the length of their steps and an abnormal paw placement during locomotion. Quantitative analysis of the locomotor behavior (under spatial and temporal cues) and after methamphetamine administration) using video recording showed significant changes in several parameters compared to control sham-treated animals, consistent with stable impairment of locomotion. Histological evaluation of the animals showed small lesions (30-60% of 3NP-treated group). These data confirm that chronic 3NP treatment leads to NMDA receptor-mediated like-lesions which are associated with stable motor symptoms.


This study examines the changes in extracellular GABA in the nucleus accumbens (NA) following quinolinic acid (QA) infusions into the striatum to determine whether 3-NP can affect the NA to provide a physical basis for behavioral changes in Huntington’s disease (HD). The NA constitutes a major functional interface between the limbic and motor systems in locomotor process, i.e., the basal ganglia (BG) has been proposed as a major pathway to modulate emotional and psychiatric disorders. Although Huntington’s disease (HD) is a neurodegenerative disorder with severe effects on striatal neuronal loss, GABA can be found in the NA is significantly decreased in HD patients with psychiatric features. Despite the common use of “decreased” as an explanation for behavioral problems in HD in clinical practice, no changes in basal ganglia have been proposed as a major pathway to modulate emotional and psychiatric disorders. Following intrastriatal analysis of QA (0.25mM) and QA (0.25mM) onto the NA, 3-NP-treated animals showed a decrease in the striatum (67% of baseline) and increase in NA (156% of baseline). On day 2, GABA returned to baseline in the NA and then declined gradually to 40% of baseline by day 7. Similar GABA slowly declined to this same level by day 7. These results show that QA perfusion of the striatum causes a slow decline in extracellular GABA in both nuclei but early transient increase in the NA. An extrapolation of such changes to the more protracted time course in HD may help to explain several locomotor and behavioral changes in humans with HD.


Excitotoxic animal models have been widely utilized to define the mechanisms involved in the abnormalities observed in Huntington’s disease (HD). Recently, 3-Nitropyruvic acid (3-NP) has been proposed as a model that closely mimics the anatomical pathology associated with HD based on the striatal atrophy following mitochondrial impairment induced by 3-NP. Since impaired oxidative metabolism has been implicated in HD patients, 3-NP seems to reflect a similar mechanism leading to striatal pathology. Very few studies have investigated the consequence behavioral pathology following 3-NP-induced lesions. In the present study, low dose systemic 3-NP-treated animals (Koutouzis et al Brain Res, in press) were observed significant hypertrophy, and accordingly suggested that this reflected a large and was abnormal HD cases. In the present study, low dose systemic 3-NP-treated animals exhibited significant hypertrophy, resulting in a plateau after the third injection (day 17), then showing significant hypertrophy from the fourth injection (day 18) onwards.

The close resemblance between systemic 3-NP’s behavioral effects in rodents and the ongoing behavioral pathology of HD in humans supports the applicability of 3-NP to HD. The present study shows that 3-NP-treated animals exhibited significant hypertrophy, resulting in a plateau after the third injection (day 17), then showing significant hypertrophy from the fourth injection (day 18) onwards. Additionally, treatment with 3-NP reversed the hypertrophy observed in the remaining HD cases. These results suggest that 3-NP-treatment is effective in reversing the hypertrophy associated with HD. QA treated animals showed increased rearing behavior, oral movements, and nocturnal activity compared to vehicle treated animals. Unlike rats receiving acute injections of QA, animals exposed to chronic QA show a more progressive development of abnormal behaviors.

Additional behavioral measures will be presented including changes in motor behavior following acute stress. Results will also show if any QA induced changes in behavior improve after the cessation of quinolinic infusion correlating with transient changes in striatal anatomy.

511.6 CHRONIC BILATERAL INTRASTRIAL DIALYTIC ADMINISTRATION OF QUINOLINIC ACID PRODUCES ABNORMAL MOTOR BEHAVIOR IN RATS. T. Bazzetti, C. Trahey, S. Ugart, J. Becker and R. Albin. Dept of Neurology and Biophysics, University of Michigan, Ann Arbor, MI 48104-1687.

We have previously reported that chronic intrastriatal dialytic administration of quinolinic acid (QA) in the rat produces selective neurodegeneration similar to that seen in Huntington’s disease (HD). In addition to neuronal loss, we have reported that chronic QA administration induces reversible changes in some areas of the forebrain. The present study shows that bilateral administration of QA using this technique, produces behavioral changes that may be analogous to those seen in HD. In the present study, rats treated with QA showed increased rearing behavior, oral movements, and nocturnal activity compared to vehicle treated animals. Unlike rats receiving acute injections of QA, animals exposed to chronic QA show a more progressive development of abnormal behaviors.

Additional behavioral measures will be presented including changes in motor behavior following acute stress. Results will also show if any QA induced changes in behavior improve after the cessation of quinolinic infusion correlating with transient changes in striatal anatomy.

511.8 TIMING THE TRANSCRIPTIONAL RESPONSE OF THE IT15 GENE (HUNTINGTIN) DURING THE EARLY STAGES OF THE NMDA RECEPTOR-MEDIATED EXCITOTOXIC CASCADE. L.R. Carlcock, K. Gutteridge, Y. Shen & P.D. Walker*. Departments of Molecular Biology/Genetics and Anatomy/Cell Biology, Wayne State University School of Medicine, Detroit, MI 48201.

NMDA receptor overstimulation produces a pattern of neuronal death within the rodent striatum similar to Huntington’s disease (HD). However, does the excitotoxic rodent model provide information that can elucidate the function of the IT15 (Huntingtin) gene in the chronic HD process? We sought to examine the transcriptional response of the rodent IT15 gene during the initial stages (1-72 hours) of the excitotoxic cascade and compare it to the IT15 gene in HD patients with psychiatric features. Despite the common use of “decreased” as an explanation for behavioral problems in HD in clinical practice, no changes in basal ganglia have been proposed as a major pathway to modulate emotional and psychiatric disorders. Following intrastriatal analysis of QA (0.25mM) and QA (0.25mM) onto the NA, 3-NP-treated animals showed a decrease in the striatum (67% of baseline) and increase in NA (156% of baseline). On day 2, GABA returned to baseline in the NA and then declined gradually to 40% of baseline by day 7. Similar GABA slowly declined to this same level by day 7. These results show that QA perfusion of the striatum causes a slow decline in extracellular GABA in both nuclei but early transient increase in the NA. An extrapolation of such changes to the more protracted time course in HD may help to explain several locomotor and behavioral changes in humans with HD.

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511.9

An attempt has been made to determine if the gene defect in Huntington's disease (HD) leads to neuronal degeneration that is a defect in mitochondrial energy metabolism or may render cells more vulnerable to endogenous levels of glutamate, resulting in excitotoxic neuronal death. In support of this hypothesis, PET studies have shown reduced glucose utilization in the basal ganglia of HD patients, while a recent NMR spectroscopy study found increased lactate concentrations in the occipital cortex and brainstem regions of HD patients (Jenkins et al., Neurology 1993, 43, 2689-2695). In addition, chronic systemic administration of mitochondrial toxins to rodents have been found to produce striatal lesions exhibiting close neurochemical and histological similarities to HD lesions (Bell, Ann. Neurol. 1992, 31, 119-130).

The aim of the present study was to investigate whether activities of electron transport enzymes and mitochondrial membrane potentials in HD patients, relative to levels in control subjects. Activities of citrate synthase and the oxidative phosphorylation enzyme complexes I, II-III, and IV, were measured in mitochondrial preparations by spectrophotometric assay procedures. Citrate synthase-corrected complex I activity was found to be significantly increased in frontal cortex of HD patients (+28%, p < 0.05), and showed a trend towards an increase in HD caudate. A trend towards decreased complex II-III activity was also evident in caudate. This study is presently being expanded to other brain regions.

(Supported by the Huntington's Disease Society of America.)

511.11

The identification and cloning of the IT15 gene and the subsequent development of high affinity antibodies raised against peptide sequences contained within the open reading frame of IT15 has provided us with the necessary reagents to isolate and purify the Huntington's disease protein (RDP). Using the affinity-purified antibodies raised against amino acid residues 1-17 of IT15, we have produced various neuronal and soluble protein subfractions isolated from rat forebrain. RDP is enriched in the synaptic vesicle-containing subfraction, but is readily dissociated from the vesicles by increasing ionic strength. The extracted RDP was fractionated by gel filtration, ion exchange chromatography and differential centrifugation on sucrose gradients and molecular weight spectrophotometry. The RDP band at 300 and 350 KDa, which cross-react with our anti-RDP Ig. We and anti-IT15 antibodies of both proteins in order to identify them. Preliminary sequence analysis indicates that the 350 KDa protein is rat brain RDP. The 300 KDa protein may be a structural homolog of RDP as indicated by the cross-reactivity to RDP antibodies. Both proteins reassociate specifically with synaptic vesicles as well as cytoskeletal elements, suggesting that RDP may play a role in neurotransmission and vesicle recycling.

511.13

The neuropathology of Huntington's disease (HD) is characterized by selective degeneration of striatal spiny neurons, a pattern of neuronal loss which can be reproduced in experimental animals by striatal injections of glutamate or NMDA agonists, suggesting that excitotoxic neurotransmission may play a role in the neurodegenerative process. Glutamate receptor antagonists may therefore ameliorate or slow HD, but such drugs (e.g. MK-801) in humans have been shown to have no beneficial effects. We conducted a randomized, double-blind, placebo-controlled tolerability trial of remacemide hydrochloride, a non-competitive NMDA channel blocking agent. Subjects were randomized to receive remacemide 250, 500 or 1000 mg/day or placebo and were assessed by standardized neurological and cognitive tests for 6 weeks, including a 1-2 week blinded staggered withdrawal of drug. The primary outcome measure was the proportion of subjects able to complete the study. Thirty-one HD patients in early to moderate stages of illness (18 months) were enrolled in the study; and twenty-nine completed treatment. One subject was terminated for non-compliance and one subject elected to discontinue drug because of nausea and vomiting (both in 600 mg group). There were no significant differences in the primary measure of tolerability between treatment arms. No severe adverse side effects or adverse cognitive effects were observed. Remacemide appears to be safe and well-tolerated in patients with HD at dosages up to 600 mg/day. Larger long-term study of the impact of remacemide on HD is warranted. (Supported by Fisons Pharmaceuticals and the University of Rochester Clinical Research Center R00004.)

511.10

Protein kinase C (PKC) is a calcium-dependent protein kinase and numerous isoforms have been found in nervous tissue. We have found that the PKCα isoform, a cytosolic isoform normally found in very low levels in striatal projection neurons, is significantly determined in striatal neurons in vivo in surviving rat striatal neurons subjected to an excitotoxic or ischemic insult. We therefore immunohistochemically examined fixed striatum from a presymptomatic Huntington's disease patient to determine if PKCα expression was increased and might reveal the early expression of the HD disease process. We found high expression of PKCα in the presymptomatic HD caudate and low expression in the control striatum. This upregulation of PKCα was largely devoid of PKCα labeling. In presymptomatic HD caudate, double-label studies showed that PKCα was expressed by 80% of the GABA (Caulin) neurons that were studied, a small number of stellate cells, but no somatostatin-/neuropeptide Y-containing interneurons. Using Calb-Immobieant to distinguish the patch and matrix compartments of caudate (low Calb neurons/pack) in a single rat and examining an adjacent PKCα labeled section, we found that a higher percentage of neurons in patch labeled for PKCα than in matrix. Our results show that enhanced PKCα expression specifically occurs in neurons destined to die in HD, implying much more than they actually die. This expression occurs first in the neurons that will die first (i.e. caudate neurons). The strongly greater expression in patch vs matrix suggests different mechanisms in matrix neurons in HD. Since the functions of PKCα are diverse, it is uncertain if the enhanced PKCα expression serves to promote or combat the HD process. Supported by NS-28721, NS-19620 and the Hereditary Disease Foundation.

511.12
CELLULAR LOCALIZATION OF HUNTINGTON'S DISEASE (HD) GENE PRODUCT: ENRICHMENT IN NERVE TERMINALS. A.H. Sharp, L. Levy, C. Schilling, A. Le, I. Hedren, S. Slodzik, T. Daniel, S. Snyder, C.A. Ross. Laboratory of Molecular Neurobiology, Johns Hopkins University, School of Medicine, 720 Rutland Ave., Ross 615, Baltimore, MD 21205.

Huntington's disease (HD) is an inherited neurodegenerative disorder with autosomal dominance and early onset of severe age-related symptoms. The causative gene contains an expanding CAG repeat presumably coding for glutamine in a long open reading frame yielding a 348 KDa predicted protein (HD Collaborative Group, 1993). The disease is characterized by degenerative neuronal loss in the basal ganglia and deep layers of the cerebral cortex which has been proposed to result from excitotoxicity. However, the mRNA for the gene causing HD is widely expressed in both central and peripheral, with no enrichment in the basal ganglia (Le et al., 1993; Strong et al., 1993). To examine the expression of the protein product, we have now developed affinity-purified polyclonal antibodies against peptides corresponding to amino acids 1-17 and 650-663 of the deduced sequence of the human HD gene product which react specifically with a 350 KDa band on Western blots. By using immunohistochemical methods, we find that the HD protein product, like the mRNA, is widely expressed and enriched in brain. Within the brain, it is more highly expressed in neurons than glia and prominent in cytoplasm, dendrites and axons. Within several brain regions, particularly the striatum, it is enriched in nerve terminals and loosely associated with synaptic vesicles and other membranes. These results are consistent with a role for the HD gene product in regulation of neurotransmitter release or reuptake and, perhaps, involvement in excitotoxicity.

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511.1


Recent advances in the understanding of the functional anatomy of the basal ganglia have led to the development of new approaches for the surgical treatment of movement disorders. The neural circuitry in the basal ganglia generates excitatory and inhibitory outputs to the pallidum via direct and indirect striatopallidal pathways. Under various pathological conditions, the balance between these excitatory and inhibitory projections can be disrupted, thus resulting in motor dysfunction. Lesions of the pallidum have therefore been proposed to eliminate the outflow of abnormal motor/sensory information. Such lesions in parkinsonian patients have been shown to ameliorate bradykinesia, rigidity, and tremor. In the present study, we have examined the effects of pallidotomy for the treatment of dystonia. Four cases of secondary dystonia resulting from different causes were treated by posterior ventral pallidotomy. Dystonia resolved from either hemiballism, cervical palsy, head injury or viral encephalitis. Each patient had a long history of spasticity and uncontrolled dynamic movements of the extremities (3 - 18 years). In addition, preoperative MRIs revealed lesions extending to the basal ganglia in each patient. MRI guided stereotactic surgeries were performed using the BKW MRI stereotactic frame. The posterior ventral pallidotomy contralateral to the affected extremity was chosen as the target site. Several radiofrequency lesions were made in the targeted pallidum after stimulation to verify the appropriate target area. Following surgery all patients exhibited marked reduction in spasticity. Postoperative MRI showed that the surgical lesions were confined to the posterior ventral pallidum. These results suggest that surgical lesions of the pallidum can ameliorate dystonia induced by a, and thus provide another support the notion of ablative ablative intervention for the treatment of basal ganglia-mediated movement disorders.

511.2


An important feature of the neuropathogenesis of HIV-1 associated dementia is neuronal loss in discrete areas of the cortex and subcortical regions. Evidence for HIV-1 infection of neurons is controversial, but soluble neuritic factors including tumor necrosis factor alpha (TNFα), produced by activated HIV-1 macrophages, may mediate neuronal death. We have demonstrated that TNFα is neurotoxic to cultures of human fetal cortical neurons in a dose-dependent fashion (Gebhardt et al., Dev. Neurosci., in press). Because TNFα has been implicated in apoptotic cell death in hypoxic-neuronal cells, we tested the hypothesis that TNFα could induce programmed cell death (apoptosis) in a neuroblastoma cell line (SK-N-MC) differentiated to a neuronal phenotype with 5 μM retinoic acid. TNFα, at a dose of 2 ng/ml, induces formation of DNA laddering in gel electrophoresis studies. Apoptotic nuclei in SK-N-MC cells were identified by an in situ method to label free 3'-OH ends of newly cleaved nuclear DNA. Apoptotic nuclei in SK-N-MC cultures can be identified 6 hours after exposure to 0.2 ng/ml of TNFα, and reach a maximum by 48 hours. At a dose of 0.05 ng/ml TNFα,- 10% of SK-N-MC cells/culture well have apoptotic nuclei. At a dose of 25 ng/ml TNFα-66% of SK-N-MC cells/culture well have apoptotic nuclei. This dose-dependent increase in apoptotic nuclei is inversely proportional to a dose-dependent (same dose range) decrease in cell viability as measured by trypan blue exclusion and enzymatic conversion of 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT). Studies are in progress to prevent TNFα-mediated neuronal apoptosis by infection of SK-N-MC cells with a recombinant adenoviral vector that expresses a portion of the extracellular domain of the TNFα receptor. (Supported in part by PAFAN/FAR and NINDS.)

512.3


Currently available scrapie-infected cell lines do not show distinct molecular correlations related to spongiform degeneration. We infected the GT1-1,1-k9 neuronal hypothalamic cell line, which secretes G0Rh in a manner that is transformed with the 1-k-A gene (NGF), with mouse BMS, prions (ScGt1-1-k9). Subclones have been in culture for over 9 months and were passage more than 40 times without any decrease in PrPC[Sc] production. Interestingly, there is a pronounced morphological difference between the ScGt1-1,k9 and the parental GT1-1-k9 cells. EM studies showed two characteristic and very prominent ultralunar changes in ScGt1-1-k9 cells, which were not detectable in GT1-1-k9 cells; a) huge vacuoles, and b) the apoptotic features of nuclear condensation and fragmentation and autophagocytosis. Extraction of Triton X 100 soluble DNA from ScGt1-1-k9 cells revealed a DNA fragmentation pattern typical for apoptosis (not detected in GT1-1-k9 cells). Although showing these very pronounced apoptotic features, over 80% of ScGt1-1-k9 cells are viable. Notably, after NGF treatment of ScGt1-1-k9 cells the DNA fragmentation was reduced. For the first time it was possible to infect GT1-1-k9 as well as mouse neuroblastoma cells (N2a) with conditioned media from ScGt1-1-k9 cells, permitting transfer of prions between mouse cell lines. Our findings present a cell culture model for the study of prion pathogenesis. The ScGt1-1-k9 cells exhibiting intracellular vacuolation and apoptotic cell death may be a useful model for neurodegeneration in humans and animals dying of prion diseases.

512.4

BIOCHEMICAL CHARACTERIZATION AND TISSUE LOCALIZATION OF PRION PROTEIN LIGANDS. B. Oesch, F. Coulie, E. Gburek, C. Bandrop, S. B. Prusiner, and S.J. DeArmond. Brain Research Institute, University of Zürich, 8029 Zürich, Switzerland and Dept. of Neurology and Pathology, University of California, San Francisco, CA 94143.

Interaction of the prion protein with other cellular components may be an important step for the function of cellular PrP (PrPSc) in the normal animal or for the infection with prions which contain an isoform of the prion protein (PrPSc). We have investigated the biochemical characteristics of PrP binding proteins resulting in the purification of a PrP ligand of 110'000 kDa (PI 110). We have used ligand blot as an essay for purification. The sedimentation properties of PrP ligands were analyzed by sucrose density centrifugation. PI 110 in postnuclear supernatant was found to sediment with high density particles suggesting that PrPSc is associated in multiprotein complexes. Purification to homogeneity was achieved by a combination of subaorticutes fractionation, ion exchange chromatography and SDS polyacrylamide gel electrophoresis.

As a second, independent line of investigation we have characterized binding sites for the prion protein in normal and scrapie-infected brain. Binding of radio labeled PrP 27-30 strongly to granule and pyramidal cells of the hippocampal formation. Competition with unlabeled PrP 27-30 revealed a Kd of 2 nM. A synthetic peptide (designated PS) corresponding to the central portion of the prion was able to reproduce the binding pattern observed with PrP 27-30. Peptide PS bound with a similar affinity and to the same sites as intact PrP 27-30. Binding sites seem to be mostly localized over cell bodies or proximal dendrites. From previous work it is known that PrP is axonally transported and located at the synapse leading to the conclusion that PrP may serve a signaling function at the synapse.

1 Oesch et al., Biochemistry 29, 5848
S12.5

Scrapie is the archetype unconventional slow infection disease. PCNA is a 36-kd, acidic, non-histone, delta accessory protein of the DNA replication machinery associated with cell proliferation. One issue is whether the astrocytosis seen in scrapie is a function of an increase in reactivity, or, if there is actual replication of astrocytes. In the current study, we used antibody to PCNA as a determinant of cell replication. Female, weanling Syrian hamsters, LWG/LAK, were inoculated intracerebrally with scrapie strain 139H or 263K or with normal hamster brain homogenates. Paraffin sections from scrapie and control brains were examined with PCNA immunostaining (ir-PCNA). We observed that the intensity of ir-PCNA in hippocampus, thalamus, hypothalamus and cortex of 139H- and 263K-infected hamsters was greater than in controls. Furthermore, by using double-staining analysis, we found that PCNA and glial fibrillary acidic protein were co-localized in brain cells. Our results suggest that the astrocytosis seen in scrapie-infected animals is, at least in part, due to actual replication of astrocytes in these animals.

S12.6

In post mortem studies of humans infected with human immunodeficiency virus (HIV), loss of large cortical neurons has been described which may, in part, explain the cognitive deficits of AIDS dementia. Feline immunodeficiency virus (FIV) produces an HIV-like pattern of disease in cats and has recently been shown to contribute to neuronal excitotoxicity in vitro. To evaluate if adult cats infected with FIV show early neurodegenerative changes which mimic the neurodegenerative changes seen in humans, we evaluated cortical cell densities in seven asymptomatic specific pathogen free cats three years after infection with FIV. Cell density in cortical layers II-V was evaluated for small, medium and large cells and compared to cell densities measured from the brains of six cats infected with FeLV. A 15-44% decrease in the number of large neurons in the frontal cortex was found. The largest decrease in neuron density correlated with low CD4/CD8 ratios. Neuronal loss was also evaluated in two FIV-infected random source cats and compared to two random source controls. A 30-34% decrease in large cell density was seen. This pattern indicates that FIV induces similar neurodegenerative changes as HIV and that these changes may begin to appear in relatively early stages of infection, before the appearance of overt behavioral or neurological symptoms.

S12.7
ANTI-MYELIN BASIC PROTEIN ANTIBODY IN PEDIATRIC AIDS PATIENTS. K.M. Weidenheim*, M. Trocchio, A. Bulkinstein and W.D. Lyman. Department of Pathology (Neuropathology) and Pediatrics (Immunology), Albert Einstein College of Medicine, Bronx, NY 10461.

Infection with human immunodeficiency virus type 1 (HIV-1) is associated with progressive neurological impairment. Characteristic white matter changes which are thought to contribute to the neurological problems include a generalized decrease in myelin, leukoencephalopathy, vascular myelopathy and corticoperiventricular demyelination. Corticoperiventricular demyelination is the most severe change in pediatric AIDS because it correlates with the progressive motor dysfunction observed clinically, and the clinically observed cognitive dysfunction may correlate with injury to hemispheric white matter during HIV-1 infection. Anti-myelin antibodies have been shown to damage white matter in other disease states, and antibodies to myelin basic protein (MBP) have been identified in adult patients with AIDS. However, anti-myelin antibodies have not yet been identified in children with HIV-1 infection. We have developed an ELISA technique which can detect anti-MBP antibody in microliter quantities of human serum. Serum samples from 4 pediatric AIDS patients with neurologic symptoms were analyzed using this system. ELISA plates coated with human MBP were incubated with test sera from 4 pediatric AIDS patients with neurologic disease. Antibody binding was detected using an ABC technique. Anti-MBP-antibodies were identified in 2 patients. Pre-absorption of sera with MBP decreased antibody reactivity. This suggests that the antibody was MBP-specific. Both children with anti-MBP antibodies had motor dysfunction, and one had neuropsychiatric impairment. These results suggest that anti-MBP antibodies are present in the serum of some HIV-infected children neurologic symptoms. These antibodies may contribute, in part, to the white matter pathology observed in pediatric AIDS. Support: U54MH6767, M446815.

S12.8
FETAL BRAIN ACCUMULATES GP120 SYSTEMICALLY ADMINISTERED TO PREGNANT MICE. J.Y. Wu T.W. Moodly, D.E. Brenneman and L.M. Squire. Dept. of Neurology, NICHD, NIH, Bethesda, MD 20892; BPR, NCI, NIH, Rockville, MD 20850.

Pediatric AIDS is often perinatally acquired and frequently accompanied by extensive neurological damage despite low levels of HIV-infected cells. The HIV envelope protein, gp120, has been shown to be toxic to neurons in culture (Nature 338:839, 1988) and to induce neuronal dystrophy, and behavioral retardation (Brain Res. 603:222,1993). The present study was designed to determine if gp120 could be transmitted from the maternal circulation to the fetal brain during pregnancy. 152 gp120 injected into tail vein mice during the 16th day of pregnancy, and fetuses were examined after 1, 24, 48 and 72 hours. The extracts of fetal brain and body were analyzed with gel filtration chromatography. The fraction co-eluting with stock gp120 is shown below in % of total CPM + S.E.M. eluted:

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hr</td>
<td>1.72±0.36</td>
</tr>
<tr>
<td>48 hr</td>
<td>1.34±0.07</td>
</tr>
<tr>
<td>72 hr</td>
<td>0.76±0.14</td>
</tr>
</tbody>
</table>

Significant amounts of radiolabeled material were found in fractions corresponding to the neurotropic gp120 fragments (Brain Res. 603:222,1993). After 2 days, approximately 0.01% of the radioactive gp120 injected into a mother appeared in the brain of one fetus. This study has shown that: 1) gp120 crossed the placental barrier; 2) although gp120 levels increased in both the fetal brain and body with time, gp120 was preferentially sequestered in the brain. These data suggest that the neurological damage occurring in pediatric AIDS may be due, in part, to fetal transmission of gp120.
3.11 PROTECTIVE EFFECT OF THYMOSIN PEPTIDES AND PHENOTHIAZINE NEUROLEPTICS ON SURVIVAL OF MICE TREATED WITH LETHAL DOSES OF ENDOTOXIN. M. Fagaras, M. Badamchian, A. Goldstein, Department of Biochemistry and Molecular Biology, The George Washington University, Washington, DC 20037

Pre-treatment with phenothiazine neuroleptics (chlorpromazine, trifluoperazine), thymosin α1, and thymosin β4, protected the mice against the lethality induced by endotoxin. Endotoxin and protective compounds were administered i.p. in Swiss-Webster mice. We investigated the molecular mechanism involved in this effect. We determined that phenothiazine neuroleptics and thymosin peptides inhibit a cascade of pathological mediators: free radicals, cytokines (interleukin-1 and tumor necrosis factor) platelet activating factor and arachidonic acid metabolites (6-Keto-PGF1α and TxB2). The effect on free radical formation was determined by measuring blood lipid peroxidation and red blood cell glutathione levels. Serum cytokine levels were measured by ELISA and plasma concentrations of arachidonic acid and metabolites were determined by platelet activating factor by RIA. Pretreatment of the mice with vitamin E, which is an antioxidant also increased survival against lethal doses of endotoxin. We concluded that free radicals are the first step in the cascade of toxic mediators induced by endotoxin. Phenothiazine neuroleptics and thymosin peptides by inhibiting free radical formation, inhibit this whole cascade which induces lethality.


Intercellular adhesion molecule-1 (ICAM-1), a cell surface receptor plays an important role in leukocyte transmigration in experimental meningitis. High levels of ICAM-1 can be measured in the CSF of patients with bacterial meningitis and provide evidence for a strong immune response, which may cause severe complications in spite of effective antibiotic treatment. We investigated the effect of systemic treatment with ICAM-1 antibodies on rCBF in male wistar rats. Meningitis was induced by intracisternal injection of pneumococcal cell wall components (equivalent of 107 cfu Streptococcus pneumoniae Pk 527, lana). rCBF was measured continuously over 6 hours with laser Doppler flowmetry. Rat ICAM-1 antibodies (TMB, Athena Neurosciences, Inc. San Francisco) were injected i.v. (1.5mg/kg) in three animals (n=3). In control animals saline was injected (n=4). There was an increase of rCBF in untreated animals 3 hours (mean±187% of baseline ± 47.8SD) and a dramatic increase 6 hours after the induction of meningitis (mean±265% ± 59%). In the ICAM-1 antibody treated group the increase of rCBF was lower 3 hours (mean± 127% ± 15%) and significantly attenuated (mean±134% ± 19, test p=0.015) 6 hours after induction. The results demonstrate that ICAM-1-1 is involved in mediating rCBF changes associated with bacterial meningitis.


To obtain evidence on an early step in the cascade of events following HSV infection in sensory ganglia, GAP-43, a protein induced by growing neurons, was examined. The hind footpads of mice were intracutaneously injected with HSV type-2 strain (9.3×106 pfu/ml or medium. At control), 5, 14 and 28 days post-inoculation (dpi), mice were fixed. Sections of decalcified spinal columns and DRGs were immunoreacted for GAP-43. Reaction product was quantified in ipsilateral (injected) and contralateral (uninjected) lumbar 4 and 5 and DRG and proximal dorsal roots using image analysis. At 14 days there was a 4- fold increase in GAP-43 immunoreactivity in the ipsilateral DRG and 8-fold increase in dorsal root. Both cell bodies and fibers were GAP-43-positive. At 28 dpi, GAP-43 was decreased in DRG, but sustained in dorsal roots. Contralateral tissues showed no increase in GAP-43, nor did ganglia or dorsal roots of mock-inoculated mice. Western analysis also showed induction of GAP-43 in dorsal roots. This result, together with selective neuropeptide alterations that we have previously shown, indicate that a sprouting response may be mounted in ganglia in reaction to herpesvirus infection.

3.15 MURINE NEUROPATHOGENIC ts - RETROVIRUS REPICATION IN MYOBLASTS AND ASTROCYTES IN VITRO T. Powere, P. Starke, A. Goldman, J. Vany, P. Bruder, A. Wawrzyn, D. Brooks Dept. of Neurology, UW Medical School, Madison, WI 53792 and Neuromolecular Research Laboratory VAMC Madison, WI 53705.

The pathogenesis of the neurodegenerative disease caused by ts- retrovirus is not fully understood. This virus can only cause spinal cord degeneration during postnatal development and not in adult mice. ts- retrovirus replication in affected animals occurs in ventricle, spleen, CNS and muscle tissue. It is unknown what the role of the replication of ts- retrovirus in muscle tissue is in the spinal cord degeneration caused by this virus. We are attempting to determine whether both muscle and CNS infection are required to produce disease. Integrated and unintegrated viral specific DNA forms are present in various tissues post infection. A novel low molecular weight, unintegrated virus specific DNA is seen in CNS tissues but not spleen or muscle.

We studied the replication of ts- retrovirus in differentiated astrocytes and muscle cells (CRL 1772) from C3H mice. Virus titers in supernatants from infected myoblasts (2x105/ml) were of the same magnitude as seen following infection of astrocytes (1x 105) at various times after infection. Cellular specific gene (actin, glut-1) expression was compared with virus specific gene (env) expression. No new low molecular weight virus specific DNA have been identified following ts- retrovirus infection in vitro. (Neurology 1994;48(Suppl 2):A358)

3.16 A PCR-BASED ANALYSIS OF HSV-1 LATENCY IN THE TRIGEMINAL GANGLION OF THE RAT. D.J. Finch*, R. Ramakrishnan and M. Levine, Departments of Neurology and Human Genetics, and GRECC, VAMC, University of Michigan, Ann Arbor, MI 48105.

Competitive quantitative PCR and RT-PCR was used to quantitate DNA and RNA from an attenuated ribonucleotide reductase (RR)-deleted herpetic simplex virus type 1 (HSV-1) mutant in the rat trigeminal ganglion after peripheral inoculation for Assessment of ganglionic scarring. Amplification of ganglionic DNA with oligonucleotide primers specific for the HSV gB gene and for the LAT gene indicated that there were approximately 4 x 106 genome equivalents per ganglion at 2, 7 and 56 days after inoculation. Amplification of ganglionic RNA with primers specific for HSV LAT indicated that there were approximately 1 x 106 LTR-pseudogenes per ganglion at 2 and 7 days post inoculation, and 1 x 104 LAT molecules per ganglion at 56 days. In situ hybridization with a digoxigenin-labeled riboprobe specific for LAT detected an average of 1-2 LAT positive cells in each positive 6 micron section of trigeminal ganglion. In situ PCR detection of HSV genomes in similar sections, using digoxigenin-labeled nucleotides with primers specific for HSV gB, identified 27-150 genome-positive cells per section. These results indicate that there are approximately 50 LAT molecules per latent HSV genome in the trigeminal ganglion, compared to 15 LAT molecules per latent HSV genome in the CNS (Ramakrishnan et al., J. Virol. 1994), but that cells with detectable LATs by in situ hybridization represent only a small proportion of those ganglionic neurons containing HSV genomes. The presence of latent HSV genomes in a large number of neurons suggests that HSV may be more efficiently in establishing the latent state than would be anticipated from previous reports.
512.17

IMPROVED BRAIN DELIVERY AND IN VITRO EFFICACY OF ZIDOVUDINE (AZT) USING A ROSEX CHEMICAL DELIVERY SYSTEM. J. Miscetti, A. Rubinstein, Z. Harish, A. Biegler, W. Anderson, and M. Brouwer. Div. of Allergy and Immunol., Albert Einstein College of Med., Bronx, NY 10461. 1Pharmos, Corp. Alachua, FL 32615 and 2Pharmos, Corp. Rehovot, ISRAEL. Improved therapy for AIDS dementia and related encephalopathies may be achieved through enhanced delivery of effective anti-retroviral agents to the brain and CNS. The goal is the chemical delivery system (CDS) which has been applied to a number of drugs including zidovudine (AZT). The current evaluations were aimed at further defining the pharmacokinetic profile of the AZT CSCS as well as establishing its in vitro antiviral efficacy against HIV in both lymphocytes and a neural cell line. AZT or AZT-CDS at a dose of 150 μmol/kg was administered to Spague Dawley rats (i.v., tail vein) for 1 hour. The AZT-CDS produced significantly higher brain levels of AZT (AUC = 425 μg.min/mg) than did equimolar AZT dosing (AUC = 135 μg.min/mg). In addition, blood levels were reduced (AZT from AZT-CDS, AUC = 583 μg.min/mL; AZT from AZT, AUC = 1580 μg.min/mL). In culture systems (CEM lymphoma and a SKNMC neuroblastoma cell line), AZT uptake after co-incubation of cells with AZT for 1 hour was minimal. By contrast, addition of the AZT-CDS to tissue cultures resulted in large increases in the uptake of the AZT-CDS followed by biochemical conversion of the CDS to its main metabolites (the AZT-CDS quaternary salt, AZT-O, as well as AZT itself). These improved uptake profiles were associated with greater in vitro efficacy. Thus, AZT-CDS at 0.5 μM was 8% more effective than AZT in suppressing p24 production (at day 3) in a lymphocyte culture infected with 6000 TCID50 of the N11 strain of HIV and 50% more effective at 0.05 μM. Furthermore, syncytia formation was completely suppressed at an AZT-CDS dose of 0.5 μM (1000 TCID50's) while AZT at the same dose was ineffective. Finally, while AZT (at 0.5 μM) is not active in reducing viral replication in an SKNMC neural cell line, the AZT-CDS provided for significant reductions in p24 expression.

MENTAL ILLNESS: SCHIZOPHRENIA

513.1

KINDLING WITH CLOzapine. J.R. Stevens* and D.D. Denney. Dept of Neurology & Psychiatry, Oregon Health Sciences University, Portland OR 97201. The apical neuroleptic clozapine improves symptoms and behaviors of many patients with schizophrenia who have failed to respond to other neuroleptics. In addition to blockade of central nervous system monoamine, muscarinic and other receptors, clozapine often causes bilateral spike-wave activity in the EEG and generalized seizures occur in 3-5% of patients treated with the drug. Acute administration of clozapine in rats induces dose related myoclonic jerks. Although considered an undesirable side effect of clozapine, this evidence for epileptogenesis could relate to the therapeutic effect of this drug. The experiments reported here demonstrate that repeated fixed subconvulsive doses of clozapine (1 mg/kg) that initially caused no behavior change, caused seizure-like activity manifest as myoclonic jerks (MJ's) after the third injection of clozapine, and reached 150 MJ's/hour by the 15th injection. These results are consistent with kindling, i.e., a progressive sustained increase of brain excitability following repeated administration of a fixed subconvulsive dose of a provocative agent. If excitation of neurons in critical brain areas is an important aspect of the therapeutic effect of clozapine and other neuroleptics, kindling with repeated fixed low dose administration of clozapine and related drugs might be effective clinically and should decrease undesirable side effects associated with conventional high dose treatment regimens.

513.2

FRONTAL DYSFUNCTION IN SCHIZOPHRENIA. J. Gold, B. Hermann, C. Randolph*, G. Chelune, M. Trencery, D. Loring, T. Goldberg, D. Weinberger, NIHMS Neuroscience Center at Saint Elizabeths, Washington, D.C. 20032. Prior neuropsychological and neuroimaging studies had implicated dysfunction of the frontal cortex in schizophrenia (SC). However, this hypothesis has not been obtained directly using a unique patient group design. In this study, we contrasted the neuropsychological performance of 66 SC patients with that of 91 patients with frontal lobe epilepsy (FLE) (36 with a left frontal seizure focus, 51 with a right frontal focus) who were drawn from 4 epilepsy surgery programs. The epilepsy patients had a mean age of onset of chronic seizures at age 12 and were refractory to conventional pharmacotherapy. All test data was obtained prior to any eventual surgical treatment. The three patient groups did not differ in age, education, or Full Scale IQ. The SC group performed significantly better than either frontal group on measures of semantic memory such as single word reading and vocabulary. The SC patients did not differ from either frontal group on measures of attention and motor speed, which we have previously found to distinguish SC from temporal lobe epileptic patients. SC patients performed significantly better than either frontal group on phonemic fluency, a task which does distinguish frontal from temporal lobe epilepsy patients, but significantly worse than the right frontal group on the Wisconsin Card Sorting Test. The data suggest the limitations of focal lesion models of schizophrenic cognitive impairment, and demonstrate differential impairment of frontal function in schizophrenia with maximal impairment in attention and working memory and a relative sparing of frontally mediated verbal fluency.

513.3

D2/D3 AND D4 RECEPTOR DENSITIES ARE NOT ALTERED IN RATS WITH NEONATAL HIPPOCAMPAL DAMAGE. Michael B. Knable, Angela M. Murray, Barbara K. Lipps, Farouq Karoum', Daniel R. Weinberger, Clinical Brain Disorders Branch, IRP, NIMH, Neuroscience Center, Washington, DC 20032. Previous studies demonstrated that rats with excitotoxic ventral hippocampal (VH) lesions postnatally develop DA-related behavioral changes. These effects were similar to some aspects of schizophrenia. We sought to determine if these behavioral effects were associated with changes in the distribution or number of striatal DA receptors. On postnatal day 7 (PD7), rats received infusions of ibotenic acid or vehicle into the VH. They were sacrificed on PD35 and PD56. Brain sections (2μm) were incubated with 10nM [3H]HOM (50.51±12.13 in control rats and 57.0±3.3% in VH lesioned rats) and [3H]raclopride. [3H]raclopride. Butaclamol (10μM) was used to define non-specific binding in both assays. Mean optical density in the dorsolateral (DLS) and ventromedial (VMS) stratum, and nucleus accumbens (NAC) were converted to fmol/mg tissue. Specific raclopride binding was subtracted from total binding to estimate D2 receptor density. There was increased binding of YM-09151-2 and raclopride in both control and lesioned rats in DLS and VMS on PD56 compared with PD35. In DLS, cell density was not decreased in control rats with VH lesions. In VH lesioned rats, there was a 50% decrease in D4 receptor density. There were no effects of lesion for either ligand or the calculated D4 receptor density in any region. These results suggest that delayed hyperdopaminergic behaviors in rats with neonatal VH lesions are not mediated by abnormalities in density of striatal D2/D3 or D4 receptors.

513.4

C-FOS EXPRESSION IN RATS WITH A NEONATAL HIPPOCAMPAL LESION IS ALTERED AFTER AMPHETAMINE CHALLENGE. Sonia M. Ulrich, Barbara K. Lipps*, Susan E. Barchus, Daniel R. Weinberger, Clinical Brain Disorders Branch, NIMH, Neuroscience Center at St. Elizabeths, Washington, DC 20032. A neonatal (day 7 after birth, PD7) ibotenic acid lesion (large size) in the ventral hippocampus results in postpubertal (PD56) onset of a variety of abnormal behaviors that have been related to corticobasal and striatal dopaminergic dysfunction. A more restricted lesion (small) does not induce behavioral hyperactivity on PD56. To further characterize this potential animal model of schizophrenia, we studied the expression of c-fos mRNA in rats with two sizes of hippocampal lesions. C-fos has been shown to be induced by catecholaminergic stimuli. Rats were previously given amphetamine (AMP) (1.5 mg/kg) twice on PD35 and PD56 and their motor activity was tested. C-fos mRNA expression was assessed using an oligonucleotide probe was assessed six weeks later 30 and 90 minutes after AMP (10 mg/kg) or saline. A significant effect of drug treatment was seen in the ventromedial (VMS) and dorsolateral stratum (DLS), in superficial and deeper bands of parietal cortex (SPC and DPC, respectively) as well as the medial prefrontal cortex (MPCF). C-fos expression was decreased in MFC in rats with a large lesion 30 min after AMP, but not in the striatum. AMP produced a significant time effect in the DPC in rats with both lesions and in DLS after a large lesion. These preliminary results demonstrate that an early hippocampal damage alters the cortical c-fos response to AMP in adult rats.
S13.5 NEONATAL EXCITOTOXIC HIPPOCAMPAL DAMAGE ALTERS Dopamine RESPONSE TO MILD CHRONIC STRESS and CHRONIC HALOPERIDOL.


The effects of neonatal (day 7 after birth, P7) ibotenic acid lesions of the ventral hippocampus (VH) on dopamine release in response to chronic stress (saline injections for 21 days) and haloperidol treatment (0.4 mg/kg for 21 days) were investigated in adult (P64) Sprague-Dawley rats. Tissue 3-methoxytyramine (3-MT) accumulation after MAO inhibition with parimypine measured by 3H-DANS was used as an index of dopamine storage. At baseline (no injection), 3-MT was reduced in striatum (ST) of lesioned rats. Repeated saline injections resulted in a further 3-MT reduction in ST, nucleus accumbens (NAc) and frontal cortex (FC) in lesioned rats, and had no effect in controls. Chronic haloperidol, compared with vehicle, increased 3-MT accumulation in ST, NAc and FC in control and lesioned rats. The 3-MT increase was enhanced in FC and NAc of haloperidol-treated lesioned rats vs controls. These data show that a developmental lesion of VH alters dopamine responsivity to environmental (chronic mild stress) and pharmacological (chronic haloperidol) challenge.

S13.7 QUANTIFICATION OF MESOPONTINE CHOLINERGIC NEURONS IN THE HUMAN BRAIN.


Karon et al. (1991) reported that schizoprenics have significantly more NADPH-diaphorase-containing, putative cholinergic, pedunculopontine nucleus neurons than controls (Psychiat. Res., Neuroimaging, 40:31-49). The purpose of the present study was to use a specific marker for cholinergic neurons, an affinity purified human antibody against choline acetyltransferase (Chat), and computer imaging techniques to map the locations of cholinergic mesopontine neurons in the human brain. Four cases were collected from the Medical Examiners Office, 22-47 years of age. Cells were mapped on one side of the brain, in 50 mm thick horizontal sections, sampled from 5.0-6.6 mm intervals (n=7-10 sections/case), and stained with 1:200-2,000 antibody concentration. The cell counts were corrected for split-cell counting error. There was an average of 14,986 ± 1,593 neurons (mean ± SEM) (11,661-17,903) within the compact and diffuse sectors of the Ch5 region, and the Ch6 region (nomenclature of Mesulam et al. J. Comp. Neurol. 281:611-630, 1989). Future studies will determine whether differences in mesopontine cholinergic neuron numbers exist in schizophrenic cases.

S13.8 EXPRESSION OF SYNTHELIN IN THE BRAIN POSTSYNAPTIC DENSITY OF THE RAT, MDX MOUSE AND A DUCHENNE MUSCULAR DYSTROPHY PATIENT.


Duchenne muscular dystrophy (DMD) is an inherited fatal disease affecting one in 3500 boys with progressive muscle degeneration. Although cognitive impairment is a common feature of DMD, mechanisms underlying mental changes are unknown. DMD is characterized by a defect in dystrophin, a 427 KD plasma membrane protein that is located predominantly in muscle, and that has recently been detected in brain. We have previously found that brain dystrophin is a component of the postsynaptic density (PSD) and is absent in the mdx mouse, an animal model of human DMD. Recently, dystrophin, a 58 KD dystrophin-associated protein, was reportedly concentrated at postsynaptic sites at sites of neuronal junction. One postulated function of syntelin is involvement in clustering of acetylcholine receptors. Co-localization of syntelin with dystrophin in muscle prompted us to examine the protein in the rat brain by focusing on the PSD. Our results, obtained by Western blot analysis using a specific antibody against syntelin, revealed that brain syntelin is an intrinsic component of PSD. Moreover, it is differentially expressed during cortical development and is expressed in a region-specific manner. Despite complete deficiency of the 427 KD dystrophin in mdx mouse and dystrophin human brain, syntelin was detectable but decreased in cortical PSDs from the mdx mouse and from one case of human DMD. Our results indicate that dystrophin and syntelin are co-localized in the PSD, but are differentially expressed in the diseased state.

S13.9 PERINEURAL NETS OF EXTRACELLULAR MATRIX: AROUND NERVE CELLS in VARIOUS NEUROPSYCHIATRIC DISORDERS. C. Neubauer1, P.R. Hof2*, J. Miklóssy3 and M.R. Celio, 1Dept of Psychiatry, Univ. of Geneva, 2Division of Neuropathology, 3Institute of Histology, Univ. of Fribourg, Univ. of Lausanne, Switzerland; 4Dept of Neuropathology, NY State Mental Ctr, New York 10028.

Perineuronal nets of extracellular matrix" (PNs) are observed around subpopulations of nerve cells in the adult brain and may control their interactions with other neurons, glial cells, and blood vessels. In the cerebral cortex, PNs are associated with interneurons containing the calcium-binding protein parvalbumin (Celio and Bilimke, Brain Res. Rev. 1994;19:128-145). In the present study, the distribution of PNs was analyzed in several areas of the cerebral cortex in the normal human brains and compared to cases affected by psychiatric (Alzheimer's disease, Pick's disease, Down syndrome and schizophrenia) and neurological disease (multiple sclerosis, epilepsy).Brains were obtained at autopsy (4-24 hours postmortem) and were visualized using Vicuna villous lectin and chondroitin sulfate proteoglycan immunohistochemistry. No obvious changes in distribution and staining intensity of PNs were observed in Alzheimer's disease, Pick's disease, Down syndrome, epilepsy, and multiple sclerosis cases. However, a decrease of PNs around neurons was found in the cerebral cortex of schizophrenic cases. This finding may reflect the fact that disruption of the extracellular matrix or loss of certain components of PNs occur in schizophrenia. Such changes in PNs around select interneuronal cell classes such as parvalbumin-containing cells may cause functional alterations in this population of inhibitory neurons in schizophrenia.

S13.10 PHARMACOLOGICAL CHARACTERIZATION OF THE BINDING OF [3H]YOHIMBINE TO D4 Dopamine RECEPTOR SUBTYPES IN BASAL GANGLIA AND CORTEX OF SCHIZOPHRENICS AND CONTROLS.

A. Murray*, T. M. Hyde, M. B. Knable, M. B. Herman, L.B. Bigelow and J. E. Kleinman, Clinical Brain Disorders Branch, NIMH, NIH, St. Elizabeths Hospital, Washington, DC 20032.

The identification of 5 distinct dopamine (DA) receptor sub-types has important implications for the D4 hypothesis of schizophrenia. The D4 receptor is particularly intriguing because it binds clozapine with high affinity. Seeman (1993), subtracting the binding of [3H]Raclopride (labels D2 and D3 receptors) from that of [3H]YOHIMBINE (labels D2, D3 and D4 receptors) for identical groups of D4 receptors, demonstrated a six-fold increase in D4 binding in schizophrenic patients compared to the controls. We characterized the pharmacology of [3H]YOHIMBINE binding and the density and distribution of D4 receptors using quantitative autoradiography in the striatum, entorhinal and prefrontal cortex, in schizophrenic brains compared to normal controls. Adjacent sections were incubated with 1 nM [3H]YOHIMBINE (1-2 nM) and 0.1 nM [3H]RACLOPRIDE (to block 5-HT1A receptors) or 0.6 nM [3H]RACLOPRIDE. Butaclamal (10uM) was used to define non-specific binding. In the basal ganglia, schizophrenia had significant increases in D4 receptors in all regions (60-80%) labeled with [3H]YOHIMBINE alone compared to controls. A similar trend was observed in the prefrontal cortex. However, the binding of [3H]YOHIMBINE was significantly decreased ([3H]YOHIMBINE binding (50%) in the subiculum and entorhinal cortex compared to [3H]YOHIMBINE alone. This suggests that [3H]YOHIMBINE binds to 5-HT1A receptors in cortical regions.
S13.11
RESPONSE OF NEUROTENSIN SYSTEMS SUGGESTS THAT THE
SHT-2 ANTAGONIST, MLD 100,907, HAS CLOAZAPINE-LIKE
ANTIPSYCHOTIC EFFECTS. G.R. Hanson*, L.G. Bush, J.W. Gibb, and
C.J. Schmidt. Dept. Pharmacol. and Toxicol., University of Utah, Salt Lake
City, UT 84112 and Marion Merrell Dow Inc., Cincinnati, OH 45215.

Drugs such as haloperidol (HA) are classified as typical antipsychotics
because of their pronounced extrapyramidal side effects and their lack of
effectiveness against the negative symptomology of schizophrenia. In
contrast, drugs like clozapine (Cloz) are considered atypical antipsychotics
because of their lack of extrapyramidal effects and their ability to relieve schizophrenia-related negative symptoms. Although
the difference between typical and atypical antipsychotics is not known, the antagonist effects of Cloz on SHT-2 receptors, an effect not shared by
HA, suggests that a serotoninergic mechanism may be important.
Based on this hypothesis, the highly selective Marion Merrell Dow SHT-2
antagonist, MLD 100,907 (MLD), may have atypical antipsychotic
effects. This was tested by comparing the responses of caudate and
nucleus accumbens neuropeptide (NT) systems (a neuroepitope dramatically altered by antipsychotic drugs) to 1, 2 and 4 doses of HA,
Cloz and MLD. These studies revealed that the pattern of changes caused by MLD, in neuropeptide-like immunoreactivity in 6 caudate
regions was much more like the effects of Cloz than HA. In contrast, the
responses of the NT systems in the anterior and posterior nucleus accumbens were very similar for all three drugs. We conclude from these
findings that (1) antagonism of SHT-2 receptors plays a critical role in the mechanism of atypical antipsychotics, (2) the MLD
compound has atypical antipsychotic effects like Cloz and (3) SHT-2 receptors contribute both to the anterior and posterior nucleus accumbens NT systems. (This work was supported by Marion Merrell Dow Inc. and
USPHS grants DA 04222 and DA 00695.)

S13.12
Reduced striatoniγral activity related to vacuous chewing movements induced by acute palmitoleic acid: a simple, reliable model for dystonia. J. N. Fergusson, M. F. Egan, S. E. Bachus, T. M.
Hyde, R. J. Wyatt, D. Gregory*, J. E. Kleinman. NIMH at St. Elizabeths, 2700
M.L.King Ave. S.E., Wash, D.C., 20032, and The University of Indianapolis,
Indianapolis, IN, 46250.

Long term haloperidol treatment produces a syndrome of vacuous
chewing movements (VCMS) and striatal neuropeptide mRNA following long
term haloperidol decanoate. M. F. Egan, S. E. Bachus, T. M. Hyde,
Elizabeths, 2700 M.L.King Ave. S.E., Wash, D.C., 20032.

Long term haloperidol treatment produces a syndrome of vacuous
chewing movements (VCMS) and striatal neuropeptide mRNA following long
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Long term haloperidol treatment produces a syndrome of vacuous
chewing movements (VCMS) and striatal neuropeptide mRNA following long
term haloperidol decanoate. M. F. Egan, S. E. Bachus, T. M. Hyde,
Elizabeths, 2700 M.L.King Ave. S.E., Wash, D.C., 20032.
MENTAL ILLNESS: SCHIZOPHRENIA II

13.17
QUANTITATIVE MEASUREMENT OF SPEM IN SCHIZOPHRENIA USING THE SEARCH COIL TECHNIQUE. B.P. Goldstein and R.C. Alexander*. Thomas Jefferson University, Philadelphia, PA 19107

Improper smooth pursuit eye movement (SPEM) in schizophrenia appears to be one of the most consistent biological correlates of schizophrenia. Most previous studies, however, have used non-quantitative methods which do not allow specification of the defects involved. In our study, 9 schizophrenic and 5 normal subjects underwent measurement of initiation and maintenance of smooth pursuit using the search coil technique. Eye movement targets were small spots of light projected onto a tangent screen 1 m in front of the subject and attention was maintained by asking subjects to detect periodic brightening of the target. For some trials involving horizontal target motion, the target was stabilized on the retina for 300 ms (open loop condition) so that the target would follow eye movements. Detailed quantification of eye movements showed considerable overlap between the gain and variability of both the schizophrenic subjects relative to controls. Further, schizophrenic subjects successfully predicted changes in target direction in the open loop condition, indicating that they can construct an 'internal model' of target movements.

13.19
NEUROANATOMICAL CORRELATES OF AN EVENT-RELATED POTENTIAL (ERP) INDEX OF AUDITORY SENSORY MEMORY IN SCHIZOPHRENIA. P.B. Wad, C. Longeagle, T. Miche, S. Andrews, G. Dixon*, & N. McCoskey, Schools of Psychology and Psychiatry, University of New South Wales, Sydney, NSW 2052 & School of Behavioural Sciences, Macquarie University, Sydney 2109 AUSTRALIA.

To investigate the functional significance of subcortical neuroanatomical changes in temporoparietal neurotransmitter function reported in postmortem and neuroimaging studies of schizophrenic patients, we recorded an ERP index of auditory sensory memory, termed mismatch negativity (MMN), from 15 DSM-III-R schizophrenic patients and 10 normal and handedness-matched healthy controls. Patients were aged between 21 and 49 years (mean age 34.3 ± 8.2 years), and included 9 females and 6 males. MMN was elicited by short tones interspersed among 50 ms standard tones during a visual distractor task. 3D volumetric MRI scans were obtained from all subjects, and Hecht's gyri (HG) and planum temporale (PT) volumes were measured using the BRAINS package (Andreasen et al., 1993). MMN amplitude at the F3 electrode was significantly reduced in schizophrenic patients, as was HG area, particularly in the left hemisphere. PT asymmetry (L>R) was significantly greater in male compared to female subjects, and there was a significant diagnosis by sex by side interaction for PT areas, reflecting significantly reduced right hemisphere PT area in male schizophrenics. MMN amplitudes were significantly correlated with HG areas (rho = 43 to 67) in both controls and schizophrenics. Supported by the NH &AMRC (Australia) and the Rebecca L Cooper Medical Research Foundation.


13.20
REGIONAL CEREBRAL BLOOD FLOW IN SCHIZOPHRENIA: CLASSIFICATION USING PIXEL-WISE DISCRIMINANT ANALYSIS. J.D. Van Horn*, K.P. Berman, and D.R. Weinberger. Unit on PET, Neuroscience Center at the 2700 Martin Luther King Jr. Ave. SE, Washington DC 20032

Regional cerebral blood flow (rCBF) differences between patients and controls have been widely reported as between-subject comparisons of normalized mean values derived from cognitive activation paradigms. Differences in activation between groups are often used to identify affected regions in the pathological subject sample. More recently, pattern recognition approaches have been used to classify subjects to regions of interest as well as pixel-based data (e.g. principle component analysis). Here we employed a pixel-based discriminant analysis approach to the comparison of schizophrenics and control subject rCBF that enables one to analyze two or more groups of subjects performing two or more tasks, maximizing the statistical difference between the groups and displaying the results as images. The advantages of this method include the ability to localize brain areas most responsible for variation between groups, while partitioning these differences into orthogonal images to reflect potential functional network differences. Additionally, the posterior probability of an individual subject’s group classification may be measured to determine, in this case, whether or not he/she demonstrates a pattern of rCBF similar to controls or to schizophrenics.

15
SYMPOSIUM: STRUCTURE, FUNCTION, AND REGULATION OF GLUTAMATE RECEPTORS.

R.L. Huganir, HHMI, Johns Hopkins Univ.; (Chairperson), P.H. Seeburg, Univ. of Heidelberg; M. Mayer, NIH-NICHHD; S. Tonegawa, HHMI, Mass. Inst. of Technology.

Glutamate receptors mediate excitatory synaptic transmission in the central nervous system and play critical roles in synaptic plasticity, neuronal development and neurological disorders. These receptors can be divided into two main classes: metabotropic receptors, which are coupled through G-proteins to intracellular second messengers; and ionotropic receptors, which are ligand-gated ion channels. The ionotropic receptors can be further subclassed into AMPA and kainate receptors and are critical in the functional synaptic plasticity and in the regulation of their role in brain function. Peter Seeburg will discuss studies on the molecular cloning of subunits for the AMPA, kainate, and NMDA receptors. This symposium will review the recent advances in our understanding of the structure, function, and regulation of these receptors and their role in brain function. Peter Seeburg will discuss studies on the molecular cloning of subunits for the AMPA, kainate, and NMDA receptors. This symposium will review the recent advances in our understanding of the structure, function, and regulation of these receptors and their role in brain function. Peter Seeburg will discuss studies on the molecular cloning of subunits for the AMPA, kainate, and NMDA receptors. This symposium will review the recent advances in our understanding of the structure, function, and regulation of these receptors and their role in brain function.

M. Mayer will present data comparing the physiological properties and allosteric regulation of native and recombinant AMPA and kainate receptors. Richard Huganir will discuss the role of protein phosphorylation of glutamate receptors in the regulation of their function and in various forms of synaptic plasticity such as LTP and LTD. Finally, Susumu Tonegawa will present studies using homologous recombination to generate mutant mice to analyze glutamate receptor function. Mutant mice that are deficient in models of synaptic plasticity such as LTP and LTD will be discussed.

16
SYMPOSIUM: GENERAL ANESTHETIC EFFECTS ON SOMATOMOTOR PROCESSING. P. Mason, Univ. of Chicago (Chairperson); E. Collins, Yale Univ.; J. Kendig, Stanford Univ.; R. Pull, Univ. of British Columbia.

This symposium will highlight our current understanding of how general anesthetics affect excitatory and inhibitory neurotransmission in specific neuroanatomical sites important in somatomotor processing. The relative contributions of actions within the spinal cord, brainstem and thalamus to the somatic unresponsiveness observed during general anesthesia will be outlined. The symposium’s aim is to increase our understanding of how anesthetics produce surgical immobility while stimulating further investigations that add to our understanding of how anesthesia might affect the brain. Paul Mason will describe the effects of diverse general anesthetics on the evoked responses and receptive field characteristics of spinal dorsal horn cells. These findings will be discussed with respect to the role of GABA and the sensory associated with general anesthesia. Kendig will review the effects of a variety of anesthetic agents on receptor-specific spinal reflex pathways. Particular attention will be given to the effects of anesthetics on the spinal cord and how these actions on a single receptor can account for somatic unresponsiveness. Mason will describe the effects of general anesthesia on pain modulatory neurons, and propose mechanisms that may account for the observed changes in neuronal firing. Predictions will be made of how these brainstem actions may affect the spinal cord. Pull will discuss the effects of inhalational anesthetics on neurons in the rat ventrobasal thalamus. Anesthetic blockade of the transitions to the thalamic firing motor characteristic of specific behavioral states may serve to interrupt somatosensory perception.

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BRAIN METABOLISM AND BLOOD FLOW:

GENERAL

THURSDAY AM

518.1

518.2

EFFECTS OF EXTRACELLULAR POTASSIUM ION CONCENTRATION
ON RATES OF PHOSPHORYLATION OF DEOXYGLUCOSE IN
CULTURED ASTROGLIA AND NEURONS.
S. Takahashi, B.F. Driscoll, M.J. Law and L. Sokoloff*. Lab. of Cerebral
Metabolism, National Institute of Mental Health, Bethesda, MD 20892.
The effects of increasing extracellular K+ concentration on glucose
metabolism in primary cultures of rat astroglia and neurons were examined.
Cells were incubated with bicarbonate buffer containing 2 mM glucose,
tracer amounts of 2-deoxy-D-[14C]glucose ([14C]DG), and various
concentrations (5.4, 28, 56 mM) of K+ for 10, 15, or 30 min followed by a 5
min incubation in [14C]DG-free buffer for an efflux of unphosphorylated
[14C]DG. Celis were digested and assayed for labeled metabolites. K* caused
significant elevations in rates of
NEURONAL
30 min
phosphorylation of [14C]DG in both
neuronal and neuronal-astroglial mixed
cultures at 15 and 30 minutes, but not
in astrogiial cultures (Fig.). Veratridine
(75 pM), which opens Na+ channels,
significantly
raised
rates
of
phosphorylation in astroglial and
neuronal cultures (132 and 116%,
respectively), and these elevations
were completely blocked by 1 mM of
ouabain, a specific inhibitor of
Na+,K+-ATPase. These results indicate
that
elevated
extracellular
K+
concentration does not stimulate
Extracellular K* concentration (mM)
energy
metabolism
in
cultured
• p<0 05. "p<0 01 V5 54 mM K*
astroglia.

DIRECT MEASUREMENT OF INTERSTITIAL GLUCOSE AND
LACTATE IN THE CONSCIOUS HUMAN BRAIN. E. Wallace. D.

Maggs. S.S. Spencer. D. Spencer. C.C. Duncan*. R.S. Sherwin and
M.J. During. Departments of Medicine and Surgery, Yale School of
Medicine, 330 Cedar St, New Haven CT 06510.

Estimates of brain glucose and lactate can be made by NMR spectroscopy.
However, the actual concentrations of these metabolites in human extracellular fluid
(ECF), and their relationship to fluctuations in the vascular compartment are
unknown. To address this issue, we used microdialysis to monitor glucose and lactate
concentrations in human brain ECF, while manipulating plasma glucose (glucoseinsulin clamp technique). Methods: Microdialysis catheters were placed bilaterally
in the hippocampi of subjects (n=4) undergoing intracerebral depth electrode
monitoring for intractable epilepsy. Studies were carried out 3 days after surgical
placement, subjects had been seizure free at least 3 days. Measurements were made
from both epileptogenic and non-epileptogenic hippocampi. Arterialized plasma and
dialysate, glucose and lactate levels were measured sequentially under plasma glucose
conditions of fasting (5.5+/-0.5mmol/l), hyperglycemia (11.5+/-0.5 mmol/1) and
hypoglycemia (3.O+-/-O.5 mmol/1). Absolute glucose ECF concentrations were
measured by net flux calculation. Results: At each level of plasma glucose, brain
ECF glucose concentrations were substantially lower than concurrent plasma levels
(-30%). In addition ECF glucose reached equilibrium 30-40 mins after plasma
glucose. In comparison fasting brain ECF dialysate lactate levels were higher than
fasting plasma lactate measurements (~x2), and did not change with plasma glucose.
Conclusions: 1. Neurons and glia are consistently exposed to glucose concentrations
that are substantially lower than those of arterial blood. 2. Brain ECF glucose shows
a delayed response to changes in plasma glucose. 3. The marked glucose gradient,
from blood to brain ECF, implies that neuronal metabolism may be unexpectedly
vulnerable to even small decrements in glucose levels, and that, in this context
lactate might serve as an alternative fuel source.

518.3

518.4

MECHANISMS UNDERLYING THE INTRINSIC SIGNAL DURING
OPTICAL IMAGING OF RAT SOMATOSENSORY CORTEX M. M.
Haglund*, D. W. Hochman, J. R. MENO, A. G. NGAI, AND H. R. WINN.
Department of Neurological Surgery, University of Washington, Seattle,
WA 98195.
Optical imaging of intrinsic signals has been accomplished in vitro in
hippocampal slices (MacVicar & Hochman, 1991) and in vivo in monkey
visual cortex (Frostig et al.,1990). The source of the in vitro signal is
primarily due to postsynaptic activation and cellular swelling, while the in
vivo signal from monkey visual cortex is dominated by blood volume
changes and oxygen delivery from capillaries.
In rat sensory cortex, sciatic nerve stimulation elicited localized
cortical potentials (N1-20 msec; P2-35 msec) that correlated with a 2550% dilation of the pial arterioles feeding the hindlimb cortex. Optical
imaging of intrinsic signals showed changes in areas fed by pial arterioles
that dilated. Cerebral blood flow/volume was uncoupled from increases
in cortical activity by topically applying theophylline (10uM; A1/A2
adenosine antagonist), decreasing pial arteriole dilation by 70-90% and
eliminating the P2 wave. Even though blood volume changes are highly
sensitive to changes in arteriole diameter, the intrinsic signals from areas
activated in controls increased in its spatial extent and magnitude in the
presence of theophylline. Topical application of CPX (1uM; 8-cyclopentyl-1,3-dipropylxanthine; A1 antagonist) blocked the P2 wave and
potentiated arteriole dilation, while intrinsic signals changes were similar
to that found with theophylline.
Uncoupling the increases in blood flow/volume from increases in
cortical activity did not effect the intrinsic signal changes. These findings
suggest that changes related to blood flow/volume are not the main
contributors of the intrinsic signal changes in our preparation.
[MMH suppt. by Klingenstein Foundation; HRW suppt. by NS21076]

EXPLORING THE TEMPORAL BOUNDARIES OF FMRI: MEASURING
RESPONSES TO VERY BRIEF VISUAL STIMULI. R.L.Savoy*.
Institute for Science, 100 Edwin H. Land Blvd, Cambridge, MA 02142; MGHNMR Center, 149 13-th St., Charlestown, MA 02129
There is little doubt that fMRI will improve the spatial resolution of functional
brain imaging; but can fMRI, on its own, yield sufficient temporal resolution to
be of value in studying the timecourse of perceptual and cognitive
processes? In the present study, we begin to address this question by
looking at the response to very brief visual stimuli...stimuli of much shorter
duration than the hemodynamic response to which fMRI is sensitive. The
goals were two-fold. First, to see if such brief temporal stimuli would elicit
measurable fMRI responses. Second, to examine the variability of response
to temporally precise stimuli, especially the latency of response.
Three normal subjects participated in the experiment. We imaged a single
7mm oblique slice through the calcarine fissure of each individual using
Gradient Echo imaging with a 1.5 T GE Signa modified by Advanced NMR,
Inc. (Surface coil, TR=400, TE=50, Flip Angle=53°)
In a given run, we presented a full-field circular checkerboard for 1000ms,
100ms, or approximately 17ms. Stimulus onset was synchronized with the
scanner. A bite bar minimized subjects' head movements both within and
between runs. This combination permitted us to average 10 runs for each
subject in each condition.
The stimuli of 1000msec duration yielded clear fMRI responses even on
single runs. The responses to the briefer stimuli were apparent in the
averaged data. In all three conditions, the response latency was consistent.
An increase in the averaged fMRI signal occurred 2 seconds after stimulus
onset. These experiments (and other experiments with TR=100) suggest a
variability of the averaged response latency of not more than 300msec.

518.5

518.6

THE ROLE OF NEURONAL NITRIC OXIDE SYNTHASE (nNOS) IN THE CEREBRAL
HYPEREMIC RESPONSE TO HYPERCAPNIA (HC) IN RATS. Q. Wang*, P.A. Pelliqrino,
V.L. Baughman and R.F. Albrecht. Dept. of Anesthesiol., Univ. of III.-Chicago,
Chicago, IL 60612
NO has been shown to play a role in the cerebral vasodilatory responses
(CVDRs) to a wide variety of stimuli, including HC. NO is produced by the action of
NOS. There are 2 constitutive NOS isoforms in the brain, the endothelial (eNOS) and
nNOS. Previous studies have used non-specific NOS inhibitors (e.g., nitro-L-arginine)
to reveal NO influences, and found, in rats, that such agents attenuated the CVDR
to HC (PaCO2 = 60-80 mmHg) by 40-80%. However, the cellular source of that NO
is not known. 7-nitroindazole (7-Nl) is reported to be a selective inhibitor of the nNOS.
In the present study, we monitored cortical cerebral blood flow (CBF) in rats, under
normo- and hypercapnic conditions, using laser-Doppler flowmetry (LDF), in the
presence and absence of 7-NI. To confirm that eNOS was unaffected by 7-NI, we
assessed the CBF responses to the muscarinic acetylcholine agonist, oxotremorine
(OXO), which activates eNOS exclusively. The experiments were performed on male
Sprague-Dawley rats (~400g, n = 9), anesthetized with fentanyl/70% N2O and
mechanically ventilated. Cortical CBF was measured via LDF. In all experiments, an
initial CBF response to 5 min HC was obtained prior to 7-NI or vehicle (corn oil)
injection. Three ml of the 7-NI solution (at 80 mg/kg, or vehicle were injected ip. CBF
responses to 5 min HC were repeated at 30 and 60 min post-injection. After an
additional 15 min, the CBF response to a 7 min iv infusion of OXO (1 ^g/kg/min) was
measured. At the end of the study, the brains were rapidly removed and the cortical
tissue was frozen and subsequently analyzed for NOS activity. 7-NI inhibited brain
NOS activity by 60% and reduced baseline CBF by 19%, but did not affect MAP or
the CBF response to OXO. This confirmed that eNOS is unaffected by 7-NI. The CBF
responses to HC at 30 and 60 min were identically attenuated (~ 50%, by 7-NI, when
compared to controls. That reduction is within the range observed by us and others
using non-specific NOS inhibitors. The results imply that a major portion of the NO
released during HC is nNOS-derived. This presumably neuronally-released NO also
contributes to the maintenance of basal CBF.

THE LOWER LIMIT OF CEREBRAL BLOOD FLOW AUTOREGULATION WITH
NITRIC OXIDE SYNTHASE INHIBITION. S.C. Jones* and C.R. Radinsky.
Cerebrovascular Res. Lab., Cleveland Clinic Found., Cleveland, OH 44195.
Current evidence indicates that nitric oxide is not involved in CBF pressure autoregulation in spite of other evidence that endothelium influences autoregulation. Because systemic NOS inhibition raises MABP, we investigated
cortical NOS inhibition with superfusion.
Four Sprague-Dawley rats (345±15 g, SEM) were anesthetized with 0.51% halothane and 70% N2O in O2 with body temperature maintained at 37°C.
The first day a cranial window was placed over a 6 mm diameter craniotomy
(no dura matter) and the animal was allowed to recover. The second day, under anesthesia, a tracheotomy was performed, permitting artificial ventilation,
and femoral arterial and venous catheters were placed. Physiological variables (MABP, PaCO2, PaO2, and pH) were stabilized. CBF was determined using laser Doppler flowmetry through the window. Animals with low CO2 reactivity were excluded. After superfusion with artificial CSF (aCSF), MABP was sequentially lowered by exsanguination to 100, 85, 70, 55, and 40 mm Hg, followed by reinfusion. After cortical superfusion with KT3 M N^-nrtro-L-arginine
(NNLA) for 103±2 m, the sequential pressure drops were repeated. The lower
limit (LL) of autoregulation was identified visually as a change in the slope of
the CBF vs MABP plot or as the maximum pressure if the plot was linear. The
LL was 59±7 during aCSF and 81 ±10 mm Hg during NNLA superfusion
(p<0.05). In one of these animals, a 3rd withdrawal sequence with NNLA plus
10-2 M L-arginine did not decrease the LL
Thus, NNLA superfusion raises the LL of autoregulation, but the increase
is not reversed by L-arginine. Although our finding is in contrast to those of
previous workers, these data suggest 1) that there is a local autoregulatory response that is undetectable with the global CBF techniques of others, 2) that
the length of NNLA exposure allowed inhibition of a NOS source that mediates
autoregulation, or 3) that the effects of systemically administered NOS
inhibitors somehow override their effect on the LL of autoregulation.
(Supported by NSF IBN 90-22190)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994


Society for Neuroscience Abstracts, Volume 20, 1994

Recently, we have identified two splice variants of the pharmacologically-defined 5-HT2 receptor, 5-HT2α and 5-HT2β, differing in the length and sequence of their carboxy termini. In rat brain, the 5-HT2α transcripts are restricted to the striatum, while the 5-HT2β transcripts are expressed in brainstem and hypothalamus, but not in cerebellum. When either receptor is transiently expressed in COS-7 cells, each of them stimulates adenylyl cyclase activity and is sensitive to the benzamide derivative cipramide. Although 5-HT2α and 5-HT2β clones exhibit very similar pharmacological profiles, some differences were noted: The expression levels of 5-HT2α were approximately 2-fold greater than the 5-HT2β clone when transiently transfected in COS-7 cells. However, when both clones were transiently expressed in parallel experiments. Despite the lower expression levels, 5-HT2β clone produced significantly greater stimulation of adenylyl cyclase relative to 5-HT2α clone. These data indicate that although 5-HT2α and 5-HT2β are splice variants and differ only at their C-terminal tails, they may nevertheless possess different coupling efficiencies to eliciting functional responses. Whether 5-HT2α and 5-HT2β couple to different isoforms of Gα and/or adenylyl cyclase remains to be investigated. Interestingly, 5-HT2α clone has 4 protein kinase C phosphorylation sites, whereas 5-HT2β clone has only 3. This additional phosphorylation site could lead to differences in the regulation of these two receptors. Alternative splicing may provide a mechanism by which functional diversity is established for 5-HT2α receptors.


Recently, we described the molecular cloning and pharmacological characterization of two splice variants (5-HT2α and 5-HT2β) of the rat 5-HT2 receptor. We now report the identification of a human 5-HT2α cDNA clone from a human hippocampal cDNA library. The amino acid sequence deduced from the longest open reading frame is 91% identical to the rat sequence, revealing 31 amino acid changes of which 11 are non-conservative, including 2 in TIM1, 1 in TIM2 and 1 in TIMV. Interestingly, the human cDNA contains one deoxyribonucleotide insertion at the 3' end of the coding region, creating a reading frame shift and introducing a stop codon 16 deoxyribonucleotides downstream in the reading frame. Due to this nucleotide insertion, the carboxy-terminal tail of the human 5-HT2 receptor is 16 amino acids shorter than its rat counterpart. The human clone displays 5 fold higher affinity than the rat clone for the 5-HT2 antagonist 1H[3H]81380B. Benzamide derivatives like cisapride, BRL-24924 and xacopride, partial agonists at the 5-HT2 receptor, all potently inhibited binding. The biggest differences between the human and the rat clones were observed with n-Me-5-HT and zacopride which exhibited at least 5 to 10 fold higher affinity for the human 5-HT2 receptor.


The 5-HT2C receptor is an abundant serotonin receptor subtype that is restricted to the central nervous system. It has been implicated in many of the physiological effects of serotonin and psychostimulant drugs, however, the lack of specific agonists and antagonists of this receptor hinders analysis of its functional significance. To study the roles of this receptor, we have generated, through homologous recombination, a mouse lacking a functional 5-HT2C receptor gene. To verify the loss of functional receptor protein, brain polyA⁺ RNA from mutant and wild type mice were injected into Xenopus oocytes. A loss of serotonin-induced depolarization was noted in oocytes injected with RNA from the mutant animals. In addition, 5-HT2C receptor immunocytochemistry revealed a loss of immunoreactivity in brain A- areas from mutant animals. Most mutant mice appear healthy and evidence no reproductive abnormalities. However, approximately 25% of males-hemizygous for this X-linked mutation display an apparent sudden death syndrome. Death is preceded by no apparent signs of illness such as loss of weight or reduced levels of activity. The age range of animals at death is from 3 to 13 weeks, with a peak incidence at 6-8 weeks of age. Studies are underway to determine the cause of death in these animals. In particular, the possibilities that deaths of mutant mice are due to seizures or cardiac arrhythmias are under examination.


The 5-HT1B receptor, which is the rodent homolog of the human 5-HT1B receptor, has been suggested to be involved in a number of physiological states such as appetite, locomotion and aggression, as well as in certain pathological states such as migraine.

In order to study the functions of the 5-HT1B receptor in the mouse, we have generated by homologous recombination, mutant mice lacking the gene encoding the 5-HT1B receptor. Such homozygous mutant mice develop, move, feed and breed apparently normally. When analyzed in a light-dark choice test and in an open field, the 5-HT1B-/- mice displayed the same behavior as their wild type littermates, suggesting that this mutation does not affect base-line levels of locomotion and anxiety. However, the hyperlocomotor and anorectic effects of the 5-HT1B agonist RU24969 (5mg/kg, i.p.) were absent in mice lacking the 5-HT1B receptors.

In the resident-intruder aggression test where isolated test mice are faced with a wild-type intruder, the mutants attacked the intruder faster and more intensely than wild type mice. This increased aggression of 5-HT1B-/- mice might be related to the fact that a class of 5-HT1B agonists termed serenics (clozapine) have antiaffective properties, and with the finding that certain impulsive aggressive behaviors are associated with deficits in central serotonin.


Although 5HT is considered as a neurotransmitter, recent findings indicate that it is also a developmental and migrational guide. In order to study the developmental function of the receptor directly, we have disrupted the 5HT2 receptor gene by homologous recombination in D3 embryonic stem (ES) cells. These ES cells express the 5HT2 receptor, that is possible to distinguish by the extent of knock-out as the result of the gene knock-out. Interestingly, inactivation of one allele of the 5HT2 receptor gene resulted in a more 50% loss in receptor binding. In parallel, transcripts encoded by the intact allele were reduced more than two-fold, up to 20% of the level measured in normal ES cells. In contrast, large amount of transcripts encoded by the truncated receptor allele were produced in the knock-out cells (1-2 fold of the expected 50% level). We speculate that the truncated mRNA is more stable than the intact receptor RNA, and the high steady state level of the truncated RNA may supply the expression of the wild type allele, a phenomenon resembling to allelic exclusion. Alternatively, the receptor allele which remained intact in the knock-out cells may have been partially imprinted. Presently, we are performing experiments to distinguish between these two possibilities. Suppression of the intact allele was observed not only in ES cells. Chimeras with high efficiency ES cell contribution displayed negligible receptor binding in tissues derived from ES cells demonstrating that suppression is maintained through development. Chimeras showed disturbances in postnatal development, probably as the result of low receptor expression. A reduction in weight gain was evident starting at postnatal day 5-10. Feeding was also reduced, particularly at the age of 5-5 weeks resulting in cachexia and eventually death.
5-HT2 receptors are members of the serotonin 5-HT family of receptors, sharing similar pharmacology, signal transduction pathways, and sequence homology. The 5-HT2A and 5-HT2C receptors contain several new regulatory elements. This new data is essential for further understanding the regulation of 5-HT2 receptor gene expression.

A BRAIN-SPECIFIC PROTEIN THAT BINDS TO 5-HT2C AND 5-HT2A mRNAs.

MOLECULAR REQUIREMENTS FOR HALLUCINOGEN ACTIONS AT 5-HT2A RECEPTORS.

TRANSCRIPTOME CONTROL OF THE SEROTONIN-2 RECEPTOR GENE.

EFFECT OF 5-HYDROXYTRYPTAMINE ON 5-HT2A RECEPTOR mRNA EXPRESSION IN P11 CELLS.
520.1


Among higher metazoa, echinoderms exhibit the most impressive capacity for regeneration. Holothurians, or sea cucumbers, respond to adverse stimuli by autotomizing and spewing out their visceral organs which are then replaced by a regeneration process. Neuronal fibers and cell bodies are known to be present within the viscera; however, previous regeneration studies do not account for the nervous component. We have used immunocytochemistry at the light microscope level (antibodies to acetylated alpha-tubulin, to the neuropeptides GFS, galanin and CCK and a novel monoclonal antibodies) and the electron microscope to describe the enteric nervous system of holothurians. We show here, that the enteric nervous system of the sea cucumber, Holothuria glaberrima, regenerates following evisceration and in 3-5 weeks is virtually identical to that of non-eviscerated animals. The regeneration of the enteric nervous system occurs parallel to that of other organ components, fibers are observed prior to the formation of the mucosal layer and the first neurons appear shortly after. The regeneration of the nervous system coincides with a high interest in view that members of the closely related phylum, Chordata, either lack or have a very limited capacity to regenerate their nervous system. Thus, holothurians provide a model system to study nervous system regeneration in deuterostomes. (Supported by grants from EPSCoR, the Whitehall Foundation, and the University of Puerto Rico.)

520.2

STIMULATION OF REGENERATIVE SPROUTING ENHANCES PREFERENTIAL MOTOR REINERVATION (PMR) T.M. Beaulieu*. Dept. of Orthopaedics and Neurology, Johns Hopkins Hosp., Baltimore Maryland 21287

Motor axons regenerating after transaction of mixed nerve preferentially reinnervate distal masseteric fibers. Collaterals of a single motor axon often enter both sensory and motor Schwann cells of the distal stump; specificity is generated by pruning collaterals from sensory pathways while maintaining those in motor pathways (J. N. Sci. 12:2730). Stimulation of collateral formation should therefore allow increased pathway sampling and greater regeneration specificity. Experiments were performed on the proximal temoral nerves of 250gm female SD rats. Forty nerves were sharply transected and sutured; 40 others were crushed with jeweler's forceps proximal to the repair site both 2 and 4 weeks before suture to stimulate collateral sprouting. Twenty nerves in each group were evaluated at 3 weeks, and 20 at 3 mos., by applying HRP to one terminal branch and Fluoro Gold to the other. The mean number of motoneurons projecting axons into the femoral sensory and motor branches at 3 weeks was similar, after routine suture (M=149, S=170), but was significantly different after crush/crush/suture (M=244, S=103, p=.001). Specificity was apparent 3 mos after routine suture (M=242, S=130, p=.001) but was greatly augmented 3 mos after crush/crush/suture (M=263, S=81, p=.001). The number of motoneurons projecting collaterals to both branches at 3 wks, the substrate for specificity generation by collateral pruning, was significantly greater after crush/crush/suture (72 vs 53, p=.01). Nerve crush before suture thus increases the number of motoneurons projecting collaterals to both sensory and motor branches, and also results in substantial augmentation of PMR. This finding mandates a search for non-invasive techniques of stimulating collateral sprouting to enhance regeneration specificity.

520.3


Recent studies have shown that dimeric interleukin 2 produced enzymatically in vitro by nerve-derived transglutaminase is cytotoxic in vitro to mature oligodendrocytes, known to inhibit axonal growth. The cytotoxic effect operates via apoptosis. Cells showed commitment to apoptosis after 3-4 h of treatment with dimeric interleukin 2. This interval could reflect a necessary rate limiting process of activation or suppression of specific genes, or modification of specific regulatory proteins. A protein that appears to act as a positive regulator of apoptotic cell death in a number of cell types is the p53 tumor-suppressor gene product. The aim of the present study was to determine whether p53 is involved in the death of mature rat brain oligodendrocytes in response to dimeric interleukin 2. We demonstrate here that the dimeric interleukin 2 induces in oligodendrocytes translocation of endogenous wild-type p53 from the cytoplasm to the nucleus, as early as 15 min after exposure. Moreover, oligodendrocytes infected with a retroviral vector encoding a p53 miniprotein expressing the dimerization site of p53 and that acts as a negative dominant inhibitor of endogenous wild-type p53 activity, are effectively protected from apoptosis induced by dimeric interleukin 2. These findings indicate that p53 is directly involved in the apoptotic mechanism induced by dimeric interleukin 2.
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P50.05

CREATION OF A NEURON INHIBITORY ENVIRONMENT TO FACILITATE AXONIC ASTROCYTIC PROTEOGLYCAN. DR. Domingues*, A. Hoke & J. Silver. Dept. of Physiology and Anatomy, Case Western Reserve University, 10900 Euclid Ave, Cleveland, OH 44106–4975.

CNS astrocytes normally support the growth and maintenance of axons. One approach to the suppression of favorable substrates which allow axons to grow on their surfaces and the extracellular matrix. We have recently discovered an in vitro method of converting the behavior of astrocytes from supplying neuronal growth-promoting environments into those which are inhibitory. This change in behavior can be activated by exposure of the astrocytes to small peptide sequences of the beta anti-AChE receptor in an osmotic form. This activation includes the localized deposition of a matrix containing chondroitin sulfate, similar to that seen at sites of reative gliosis after trauma in vivo. Amongst the various proteoglycans synthesized and carried by astrocytes, we have found that the experimentally induced inhibitory environment is mediated by the upregulation and deposition of a specific chondroitin/heparan sulfate proteoglycan. This proteoglycan has properties inhibitory to axonal outgrowth when combined with other molecules of the extracellular matrix. These inhibitory properties can be experimentally reversed by removal of chondroitin sulfates glycosaminoglycans from the matrix created by activated astrocytes, but not by enzymatic removal of other glycosaminoglycans. Affinity purification of this proteoglycan has revealed that it is highly glycosylated and carries the carbohydrate epitopes 70kD, CS-56 and 38kD. Data from molecular weight estimations, preliminary peptide analysis and amino acid composition determinations suggest that this may be a novel proteoglycan to the CNS, which is upregulated in areas of reactive gliosis.

P50.07

SHORT- AND LONG-TERM DEGENERATIVE CHANGES IN PROTOGELICIAN CORD NEURONDED BY THEIR ABILITY TO INHIBIT AXONAL REGENERATION. A. K. Gulati* Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA 30912.

The present study was designed to define the stenotic and chronic changes in peripheral nerve undergoing degeneration and their ability to support axonal regeneration after transplantation. Peripheral nerve were degenerated in vivo for a period of 6, 12, 24, and 48 weeks. The nerve segments were analyzed for axonal regeneration and for axonal outgrowth after transplantation in an immunologically injured host. During early phase of degeneration the injured axons and the associated myelin is broken down and absorbed by proliferating Schwann cells and infiltrating macrophages. The Schwann cell basal lamina persists throughout degeneration, however, were progressively reduced in size and at 12 months were reduced. These long-term degenerated segments were reduced in diameter as compared to short-term degenerated nerves (2 and 4 weeks) that exhibited increased diameter. In order to study axonal regeneration through degenerated segments, 6 cm long segments were transplanted in grafted and distal host nerves were analyzed to determine axonal regeneration. Axonal regeneration was rapid and by 12 weeks many myelinated nerves were observed in host nerve, with some reinnervation of target muscle. Although many neural degenerated segments were observed in the 46-week degenerated transplant, the nerves remained small in diameter and the axons were thinly myelinated. In addition buildup of connective tissue was observed in all grafts. The results show that, short-term degeneration is beneficial as compared to long-term degeneration in supporting axonal regeneration (supported by NIH grant NS-28543).

P50.09


Recovery from spinal cord crush has been studied in neonatal (P5-7) and postnatal (P14-20) S.Amercian opossums (Monodelphis domestica). Mothers were anesthetized with intraperitoneal pentobarbium; the young, still attached to the mother received additional inhalation Meflocone anesthia. Spinal cords were crushed at the upper thoracic level via a skin incision using fine forceps. Completeness of crush was assessed by application of a minus one perioperative stimulus to the hind limbs below the lesion both at the time of lesion and on the day following; absence of a forelimb response was taken as indicating a complete lesion. Control sites were unoperated. Various recordings were made at operated and control animals at different intervals after surgery up to and beyond waking (P60). Spinal cords were removed under terminal anesthesia at different times after operation and in control. Conduction across the crush was tested for in vivo by electrical stimulation of the central end of the cord and recording caudally. In 12 neonates the spinal cord was isolated 24-48h after crushing. The 2 out of 12 showed complete block of conduction. In the other 2 the amplitude of the mass action potential was less than 1% of controls. Conduction across the crush by recovery was 5 to 10 days post crushing (n=16). Bosl's fixed spinal cords were examined morphologically using silver staining and examination of thick (30-50um) sections using an Edge Scientific Stereoscopic. Complete crush at P5-7 was followed by fibre growth across the crush by 5 to 10 days after operation. Substantially normal locomotor function was present towards the time of waking (P60-69). In neonates operated at P14-20 recovery of conduction did not occur.

Supported by the Ramaciotti Foundations, Sydney.

P50.10


Fusiform outgrowth of neurons has been shown to occur into and beyond the site of a crush in the spinal cord isolated from a 5 day old opossum. We have now analyzed repair at various stages after injury, by light and electron microscopy. During the first 1-3 days, the site of the lesion as well as rostral and caudal segments were characterized by identifiable elements. After 5 days in culture axons and growth cones unaccompanied by glia entered the lesion. Growth occurred along the basal lamina of the pia mater on which synaptic structures were made. By contrast in lesioned spinal cords in culture we observed 12 days after injury, less and more poorly formed elements. By 5 days in culture axons and growth cones unaccompanied by glia entered the lesion. Growth occurred along the basal lamina of the pia mater on which synaptic structures were made. By contrast in lesioned spinal cords in culture we observed 12 days after injury, less and more poorly formed elements. By 5 days in culture axons and growth cones unaccompanied by glia entered the lesion. Growth occurred along the basal lamina of the pia mater on which synaptic structures were made. By contrast in lesioned spinal cords in culture we observed 12 days after injury, less and more poorly formed elements.
S21.1
HUMAN OSCILLATORY BRAIN ACTIVITY NEAR 40-Hz COUVRIES WITH COGNITIVE TEMPORAL BINDING.

M. Joliot 1, U. Ribary* and R. Langla. Center for Neuroimaging, Dept. of Physiology and Biophysics, New York University Medical Center, New York, NY 10016, USA. *THCP, DRCP/CEA, Orsay, France.

The minimal time interval required to identify auditory stimuli as separate correlated cycles in the 40-Hz magnetic signal in the human brain, in order to test the hypothesis that 40-Hz activity may represent a temporal binding by the C.N.S.

Neurophysiological recordings of coherent oscillatory activity near 40-Hz were obtained from auditory areas of the right hemisphere from nine healthy adults, using a 37-channel Magnetoencephalography (MEG) system (BTF). Subjects attended to auditory stimuli presented in blocks consisting of one or two clicks (10 kHz, 60dB SPL) presented binurally at varying interstimulus intervals (3, 6, 9, 12, 15, 18, 24 or 30 ms). 1000 epochs were recorded for each block with inter-pair intervals of 130±10 ms, the transient responses were averaged and filtered between 20-50 HZ. The average response to a single stimulus consisted of a 2.5 oscillatory cycle 40-Hz response. A set of two stimuli were then presented and the perceptual threshold for identifying the stimuli as two events was established. Experimental and modeling results indicate a stimulus-interval dependent response with a critical time interval of 12-15 ms. At shorter intervals, at which the subjects reported the perception of only one auditory event, only one 40-Hz response was observed. As the interval increased a second 40-Hz wave appeared, abruptly. This second 40-Hz correlated significantly with the recognition by the subjects that two distinct auditory stimuli had been delivered.

These results indicate that oscillatory activity near 40-Hz represents a neurophysiological correlate to auditory temporal processing. It also supports the view that 40-Hz activity relates not only to primary sensory processing, but also to the temporal binding underlying cognition.

S21.3
ELECTROPHYSIOLOGICAL AND BEHAVIORAL SIGNS OF HEMISPHERIC ASYMMETRIES OF ATTENTION IN A “SPLIT- BRAIN” PATIENT. A. M. Proverbio 1, A. Zani 2, G. R. Manzan 1, M. S. Gazzaniga 1 1 Center for Neuroscience, University of California, Davis, CA 95616. 2 Institute of Psicologia del CNR, 00137 Rome, Italy.

Neuropsychological data have shown that the two cerebral hemispheres differ in the control of spatial attention. The present study investigated hemispheric asymmetries of attention in a left-lesioned subject who underwent incomplete resection of the corpus callosum, thereby disconnecting the cerebral hemispheres at the cortical level. Simple reaction times (RTs) and Event-related potentials (ERP) were recorded to lateralized visual stimuli in the patient J.W., and in an age-matched normal control subject (both right-handed). The ERPs were recorded on a Macintosh Quadra 710 computer and labeled using the non-cerebral stimuli as reference. The subject's eyes were monitored by means of an infrared video-camera and horizontal and vertical electro-oculogram. Stimuli were randomly presented in the left or right visual hemifield of the left eye. The ERPs were recorded from the occipital surface (Oz) and the parietal area (P3) at Pz. In contrast, in the split-brain patient RTs were 20 ms slower for more eccentric stimuli in the LVP, but no effect of eccentricity was evident for RVP stimuli. The left hemisphere showed a strong response to RVP stimuli, while LVP stimuli elicited a much greater response over the contra-lateral hemisphere. The subject showed previous theories (e.g., Kinsbourne, 1987) that the right hemisphere allocates attention broadly over left and right hemisphere, while the left hemisphere is strongly biased toward the right.

Supported by the DFG (Pu 97/2).

S21.4

Words and pronounceable pseudowords are physically similar, but invoke different cognitive processes. Gamma-band responses of the brain are related to cognitive processing, these stimuli may induce distinct patterns of gamma-band responses. Using MEG, we recorded the corresponding hemispheric responses. We used two different stimulus conditions: presentations of single words and pseudowords. The gamma-band activity in the left hemisphere was larger for pseudowords than for words, while gamma-band activity in the right hemisphere showed the opposite pattern. The results suggest that gamma-band activity in the MEG is sensitive to the different cognitive processes that underlie word generation and pseudoword generation.
S21.5 LOCALIZATION OF SPONTANEOUS OSCILLATORY CORTEX ACTIVITY FROM MAGNETOENCEPHALOGRAPHIC DATA RECORDED DURING THE IMAGINATION OF MOVEMENT AND SILENT SPEECH. Claudia D., Tebse* C., Stöferle A., D. Jancke, M. Hajo Low Temperature Laboratory, Helsinki University of Technology, 02150 Espoo, Finland. Risto J. Ilmoniemi BioMagn Laboratory, Helsinki University Central Hospital, 00290 Helsinki, Finland.

Does mental imagery in the absence of external stimuli alter the spectral characteristics of spontaneous oscillatory activity in humans? We measured spontaneous oscillatory activity in patients with 12-channel whole-scalp magnetoencephalographic (MEG) array. The subjects performed two contrasting tasks: the imagination of the self-performance of a motor activity and the silent generation of a chain of words. We developed a novel analysis method, frequency-domain signal-space projection (FDSPP), to analyze the Coherence of oscillatory activity at specific cortical sites from this data. Although intersubject differences were significant, highly reproducible spikes of oscillatory activity were observed within subjects and hemisphere and task differences in which showed variability in the spectral features with task including variations in amplitude and power, state-dependent changes in the frequency of sharply defined peaks. FDSPP analysis may also be applied to magnetoencephalographic (EEG) data. Objective evidence of the constancy of the state of a subject obtained from FDSPP may facilitate the interpretation of psychophysiological data. FDSPP analysis of rhythmic EEG and/or MEG data recorded during epileptic activity and sleep may lead to the localization of sources of activity in specific neuronal populations.

S21.6 THE EFFECTS OF BROMOCRIPTINE, A D-2 Dopamine Receptor AGONIST, ON THE COGNITIVE ABILITIES OF HUMANS SUBJECTS WITH DIFFERENT WORKING MEMORY CAPACITIES. J. Y. Kimbara, M. D'Esposito and M.J. Farah. Department of Psychology, Carleton Univ., Ottawa, Canada, and Pittsburgh, PA 15213; Dept. of Psychology, Univ. of Pennsylvania, Philadelphia PA 19104.

Recent research in individuals suggests that the prefrontal cortex (PFC) subserves working memory (WM), and that dopamine plays an important role in the PFC WM system. In order to provide a test of this hypothesis, and to investigate the role of dopamine and WM in a variety of cognitive tasks, we administered a battery of tasks to normal human subjects twice, on and off bromocriptine (BC). This resulted in a dose dependent response supported by a double blind procedure. The battery of tasks included: working memory tasks (spatial working memory, verbal working memory, and the fan effect paradigm), other tasks to depend on D-2 (PFC) (The Wisconsin Card Sorting Task, Stroop Task, a context memory task, and a dual task paradigm), and a control task (visual search) not hypothesized to depend on WM or PFC. Although there was no significant main effect of bromocriptine over all subjects; those with lower WM capacity benefited from the drug on both WM tasks and other PFC tasks and, more surprisingly, those with higher WM capacity were reliably impaired by it on these same tasks. No effects of bromocriptine were observed for the visual search control task. These results demonstrate a selective effect of bromocriptine on PFC tasks, which depends on subjects WM capacity.

S21.7 DISCORDANCE FOR PLANUM TEMPORALE ASYMMETRY IN HANDEDNESS-DISCORDANT MONOZYGOTIC (MZ) TWINS. H. Steinmetz, H. Rietzschel, H. Neukirch, G. Schlaug, Y. Huang and L. Jäncke

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Studies comparing biological with adoptive families have shown that handedness is not exclusively determined by prenatal factors. It is unclear whether these factors are genetic or non-genetic. Using in vivo magnetic resonance (MR) morphometry we measured the degree of planum temporale (PT) asymmetry $A_{PT}=(R-L)/(R+L)$ in 20 MZ twin pairs concordant (10 right-handed pairs) or discordant (10 pairs) for handedness reports. Handi-ness reports by birth complication, neurosychiatric illness, learning disability or failure in elementary school (3 MR observers blind for twins vs. 60 controls, and right vs. left hemisphere). As previously described for singletons, right-handed twins showed leftward PT asymmetry (p<0.05) whereas the right-handed co-twins, as a group, lacked asymmetry (p>0.10). Intraclass correlations were low (R<0.25). This discordance for structural asymmetry can be accounted for by models assuming cleavage of an intrinsically asymmetric blastomere or differential action of other non-genetic factors within MZ twin pairs in utero. The findings confirm a coupling of lateralized brain structure and function. At least in MZ twins, early epigenetic factors must play a role in laterality development.


Despite inferential evidence from cognitive and other behavioral studies and from animal experiments, there is little direct information about the effects of hormones on human neurophysiology during cognition. We used the oxygen-15 water PET method to measure regional cerebral blood flow (rCBF) in four women with menstrual-related mood disorder (MRMD) and four controls during three pharmacological conditions: 1) ovarian suppression induced by gonadotropin-releasing hormone (Lupron), 2) Lupron plus estrogen (E), and 3) Lupron plus progesterone (P). During each drug condition subjects were scanned at rest and while performing the Wisconsin Card Sorting Test: a simple matching task. Absolute CBF (ml/min/100g) was determined pixel-by-pixel, and the three PET data sets were each registered to a single MRI scan on which regions were drawn. Global CBF was lower in the patients than in the controls during the resting Lupron baseline (45 vs. 55, p<0.04) and tended to increase with P and decrease with E for the group as a whole. The region most affected by hormone treatment was the superior left anterior cingulate (ANOVA drug effect F=4, p<0.05), which was increased by P and decreased by E during the tasks despite unaltered performance scores. These data provide direct insight into the effects of gonadal steroids on brain function during cognition and may also suggest a neuroendocrinologic characteristic of patients with MRMD.


We sought to define the cognitive and physiologic basis for semantic memory dysfunction in Alzheimer's disease (AD). Cognitive measures were used to identify the semantic memory deficit in patients with probable Alzheimer's disease (pAD) during comprehension and expression of words and pictures. An equilibrium technique used 15O-H2O to quantify regional cerebral blood flow (rCBF) in individual patients and controls during a semantic category memory task (identifying exemplars of a familiar semantic category). An individualized, anatomically-based atlas was assigned to each subject's MRI localized cCBF activation. Cognitive assessments revealed that several pAD patients had a segmental semantic memory deficit. These patients demonstrated characteristic difficulty understanding and expressing word and picture information about a target category that could not be attributed to a category related memory specific cognitive limitations. PET assessment revealed that individual control subjects consistently recruited left angular gyrus during the PET semantic challenge while pAD subjects did not. pAD patients failed to recruit left angular gyrus during a semantic challenge while PET measured cCBF. Instead, pAD patients recruited right inferior parietal structures. pAD subjects failed to recruit left angular gyrus during a PET semantic challenge. We hypothesize that the inability to access memory by integrating information from multiple, material-specific and modality-specific sources.

S21.10 IMPAIRED RECOGNITION MEMORY IN AGING IS ASSOCIATED WITH REDUCED ACTIVATION OF HIPPOCAMPUS AND FRONTAL CORTEX. C, Grady* A.R. McIntosh, B. Horvitz, LG Ungerleider, P. Tootell, M. Pentz, IV, Haxby. Lab. of Neuroscience, NIA, Lab. of Neurophysiology, NIMH, and Lab. of Psychology and Brain Pathology, NIMH, Bethesda, MD 20892.

We measured rCBF in 10 young (25 ± 2.5 yrs) and 10 old (69 ± 6 yrs) healthy subjects using positron emission tomography and [15-O]water during learning of and recognition memory for faces. Three tasks were carried out by all subjects in the following order: learning, face matching, and recognition. Old subjects performed significantly worse than young subjects during the recognition task (ANOVA: p<0.001). During learning, compared to face matching, young subjects showed increased rCBF (p=0.001) in the right hippocampal region, left inferior frontal and medial frontal cortex. Old subjects did not show significant activation of the hippocampus during learning. They did show activation in left frontal cortex (p=0.001), but in a location different from the one seen in young subjects. During face matching, young subjects had rCBF increases in right frontal and parietal cortex, and anterior cingulate (p<0.001), and the old subjects had rCBF increases in right frontal cortex (again, in a different location). The results support the hypothesis that experiments that study frontal activation during recognition memory, and provide evidence for the role of the hippocampus in stimulus encoding. In addition, they suggest that reduced recognition in older subjects is due, at least in part, to a failure to adequately activate the hippocampal area during learning, and thus to adequately learn the list of faces. Alterations of rCBF in prefrontal cortex also appear to play a role in age-related memory impairment.
DEGENERATIVE DISEASE: ALZHEIMER’S—MECHANISMS OF DEGENERATION

S21.1

LOCALIZATION OF LANGUAGE OPERATIONS WITH H2/18 PET: INDIVIDUAL DIFFERENCES IN FUNCTIONAL ANATOMY AND THEIR RELATION TO IMAGE SMOOTHING. S.Y. Bookheimer and T.A. Zelazo. Brain Mapping Division UCLA, Los Angeles, CA 90024 and National Institute on Aging, NIH, Bethesda, MD.

Sixteen normal volunteers underwent positron emission tomography while performing simple language tasks (reading words and naming objects). Images were stereo-normalized and smoothed using Statistical Parametric Mapping (MRC Cyclotron Unit) to an effective PWHM of 12 or 21 mm. Although the same critical threshold was used in both analyses, the results differed markedly: activation in left inferior frontal (IFG) cortex (Broca’s area) was identified only with the narrow filter while significance in cerebellum, striatum and extrastriate cortices were detected with both filters. However, spatially of the most common brain regions were identified in these regions only with the narrow filter.

A second experiment with left-handers who are known to have greater spatial variability in language organization confirmed the difficulty in obtaining reliable anterior but not posterior results. High between-subject variability in the structural and functional anatomy should favor broad band filters, which are more sensitive to changes occurring over a wider spatial extent; inferior frontal language areas are among the most variable cortical structures. Our data suggest that the optimum smoothing kernels will differ as a function of the known anatomic variability. Interpretation of PET language data is critically dependent on how this variability is treated in image analysis.

S22.1


A series of 5 studies investigate topographic aspects of nonlinear EEG dynamics and their changes with information load, aging, and dementia. Major findings are that dimensional complexity (DCX: estimated correlation dimension) topography corresponds to EEG patterns of different cortical areas; some areas are more "chaotic" than others. Topographic patterns change in response to stimulation, and DCX offers a single-variable approximation of information load. Increasing visual information load increases DCX, especially over occipital areas in young subjects, but this effect is reduced in aging and nearly lost in dementia. Addition of nonlinear measures to traditional linear (FFT) measures improves the discrimination of demented patients from controls. For both types of measures, nonlinear neural net prediction offers significant advantages over linear analysis methods. Thus, nonlinear EEG measures and analysis methods offer unique perspectives for understanding both normal and pathological aging.

S22.2


Functional neuroimaging using MRI techniques (MRI) have unique capabilities for mapping the physiological correlates of behavior; their potential clinical applications are less clear. In this study, we evaluated the utility of a fast 5-dimensional (3D) gradient-echo technique in a patient population with various neurologic disorders. This novel method has the capability to scan the whole brain on a conventional 1.5 T MR scanner in only two seconds. Nine normal volunteers and patients with mild to moderately severe dementia of the Alzheimer type (DSM-III-R diagnosis), one patient with a subacute cortical infarct in the left middle cerebral artery distribution and one patient with a high grade lymphoma were studied in the resting condition. Scans were performed on a conventional 1.5 T GE/SIGNA MR scanner. Modified (FS) BURST MRI was performed during intravenous bolus administration of Gd-DTPA (0.15 mmol/kg). 3D datasets were acquired with an effective spatial resolution of 4.3x3.3x4mm within 2.5 seconds. 3D maps of relative cerebral blood volume (rCBV) and bolus arrival time were created by fitting a synthetic curve to the intensity time course on a pixel by pixel basis. The rCBV maps of the Alzheimer’s patients revealed focal areas of hypoperfusion in a frontotemporalparietal distribution identical to their SPECT perfusion scans. In the patient with the subacute infarct, the transit time maps showed arrival time delays of 5-7 seconds within and around the infarct clearly confirming the diagnosis of left middle cerebral artery occlusion. In the patient with CNS lymphoma decreased CBV around the tumor correlated with a mass effect. No focal defects were identified in the normal controls. These results illustrate the technique’s potential as a clinical tool in the diagnosis and management of various brain disorders. This technique can be added to routine clinical MR scanning, requiring additional imaging time of only 3-5 minutes.

S22.3

ANALYSIS OF STAGED AUTOPSY CASES UNCOVERS PACE OF ALZHEIMER’S DISEASE-RELATED NEOVASCULAR CHANGES. J. Bohl (1), H. Muller (2), H. Braak (2). (1) Abt. Neuropathologie, J.Gutenberghospital Bonn-Kleinkamp (2) ZMorph., J.W. Goethe University, 60590 Frankfurt; Germany.

The speed of progression of Alzheimer’s disease (AD)-related neurofibrillary changes is unknown. This is because of the impossibility to histopathologically follow one and the same individual over decades of its life. The present approach is based on a recently introduced staging system which differentiates between six stages of AD-related neurofibrillary changes and analyzes a staged sample of 887 autopsy brains. The time needed to attain respective stages of pathology for 5% of a given cumulative sample is determined. The resulting 5%-percentiles are a measure for the average pace by which the disease-related changes progress assuming that the underlying stages represent a sequence of events and do not independently “pop-out”. Advancing age and the prevalence of AD-related changes of a given stage show a nearly perfect positive correlation with only slight acceleration above the age of 65. Statistically, it takes at least 16 years to shift from stage I to stage II, 14 years from stage II to III, 13 years from stage III to IV and 5 years from stage IV to V (≈AD) for 5% of a given cumulative sample. Obviously, the deep roots of AD-related neurofibrillary changes can be traced about 50 years back and thus even extend into adolescence. The unveling of the duration of the shift between selected stages provides a powerful tool for epidemiological studies. A possible risk factor should shorten this period whereas beneficial influences would result in a delayed shift even if the considered cohort dies “too early” for the full development of AD.

S22.4

LAYER-SPECIFIC DENDRITIC ALTERATIONS OF HIPPOCAMPAL GABAERGIC NEURONS SUGGEST TRANSEUNERAL CHANGES IN ALZHEIMER’S DISEASE. S. Munch, R. Nitsch* and T. G. Ohm Center of Morphology, University Clinic Frankfurt, F.R.G.

Neurons in layer II and III of the entorhinal cortex are the first to exhibit neurofibrillary changes in Alzheimer’s disease (AD). In this early stage of the disease, the target area of axons arising from these neurons, i.e., the hippocampus, remains devoid of these changes. Experimental studies have demonstrated layer-specific transneuronal changes on dendrites in the termination zone of entorhinal fibers, e.g., the outer zones of the dentate molecular layer. The present study was designed to unravel whether similar transneuronal dendritic changes occur during the spread of neurofibrillary tangles from the entorhinal cortex to the hippocampus as shown in a staging analysis by Braak and Braak (Acta Neuropatol. 82, 239, 1991). We analyzed the hippocampal formation of 33 staged Alzheimer cases obtained from autopsy. The entire dendritic tree of parvalbumin-immunostained hippocampal GABAergic neurons was monitored using an interactive neuron tracing system. The course of population is known to remain devoid of neurofibrillary tangles even in severe AD. The quantitative analysis revealed a statistically significant reduction of the mean dendritic length, the segment number of main branch complexity and the percentage of tangle-bearing dendrites in the course of AD. These changes were confined to the apical dendritic tree which extends into the termination zone of entorhinal fibers. In contrast, basal dendrites extending into the hippocampus remained unchanged in their morphology. Our data demonstrate that layer-specific dendritic alterations in hippocampal GABAergic neurons appear together with the spread of histopathological changes in AD. This suggests the occurrence of transneuronal changes on the hippocampal targets of entorhinal neurons. (Supported by the DFG. NI 344/1-1, NI 344/5-1, and OH 484/1-1.)
522.5
DYSTROPHIC PERIKARYA AND NEURITES INTERSPERSED WITH NORMAL NADPH DIAPHORASE-NITROGEN MONOXIDE SYNTHASE (NOS)-CONTAINING NEURONS (NOSN) IN THE CEREBRAL CORTEX OF PATIENTS WITH NONALCOHOLIC STEATOHEPATITIS (NASH) AND ALZHEIMER DISEASE (MND), BUT NOT WITH MND WITHOUT DEMENTIA. R.O. Kuljis* and R.I. Schipper. Dept. Neurology and Pathology, The University of Iowa and VAMC, Iowa City, IA 52242.

Many adult patients with MND develop cognitive impairment, which may anticipate motor manifestations, and that is most frequently not attributable to supernormal conditions such as dementia. We therefore asked whether such cognitive deterioration in patients presumed to have an insult selective to motor neurons remain elusive. In the course of a series of studies on the role of NOSN in neurodegenerative diseases, we discovered that those nerve cells exhibit clearly dystrophic features in MND. Five patients with sporadic MND were studied, two with neurophysiologically verified dementia (MND+d, both 65 years old; 2:13 hours of postmortem autolysis) and one without dementia (MND-; 64-68 years old; 2:18 hours of autolysis). Tissue blocks from nine primary sensor, motor, association and limbic areas of the neocortex were sectioned frozen and reacted for NADPH diaphorase histochemistry, which reveals NOSN and their neurites. A conventional histopathologic analysis failed to show a cause for the cognitive symptoms in MND+d, but verified the clinical diagnosis of MND in all cases. Cases with MND+d, but not those without dementia, exhibit numerous markedly dystrophic NOSN perikarya and neurites, interspersed with normal NOSN in all cases examined. An additional series of control tissue sections from four age-matched and two younger patients — with a wide range of autolysis intervals and fixation protocols — failed to reveal similar dystrophic changes, indicating that they may be selective to MND+d. These observations suggest that cognitive impairment in MND+d may be related to a pancellular deterioration to the ubiquitous NOSN network, indicating a novel role for these neurons in neurodegenerative disease. Supported by PHS grant NS29856.

522.7
DISTRIBUTION OF NGF IN THE NORMAL AGED HUMAN BRAIN AND IN ALZHEIMER'S DISEASE. S.A. Scott* 1.Weigent, and K.A. Crutcher. Department of Neurosurgery, University of Cincinnati School of Medicine, Cincinnati OH 45267-0515.

Using the two-site ELISA, both the rodent and human NGF have been examined for NGF content with high levels found in the hippocampus and neocortex. Recent data from our laboratory suggest a similar distribution in the aged human brain (mean 74 pg/g), and, moreover, that the levels are comparable to those observed in other species. The highest levels of human NGF are within the dentate gyrus (1200 pg/g) whereas the lowest are found in the medial temporal cortex (0.06 pg/g). Between these extremes, neocortical areas contain 300-500 pg/g NGF with little variation among the principal gyri, although the temporal and cingulate gyri contain the highest levels. The basolateral amygdala and the nucleus basalis of Meynert are rich sources of NGF (< 700-800 pg/g). Other areas with notably high levels include the choroid plexus (> 1000 pg/g), entorhinal cortex (750 pg/g), medial thalamus (640 pg/g), and neocortex (450 pg/g), whereas the pineal gland, olfactory bulb/tract, hypothalamus/mammillary bodies, and globus pallidus contain generally lower levels (< 350 pg/g). This broad spectrum of NGF localization in brain, together with the known distribution of high-affinity trkA receptors, suggests that brain NGF is not localized simply in relation to the basal forebrain cholinergic system.

NGF levels were at least moderately higher with Alzheimer's disease in all regions examined (frontal and occipital poles, amygdala, putamen, hippocampus, cerebellum, superior temporal gyrus and inferior parietal lobule), although the distribution was similar to that in aged controls. Supported by NIH N535140 and AG05605.

522.9
ALZHEIMER'S DISEASE: PATHOGENESIS. EVIDENCE OF INCREASED PROPOXIDANT ACTIVITY IN THE INFERIOR TEMPORAL LOBE. A.M. Palmer* and M.A. Burns. Departments of Psychiatry and Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

The concentration of a product of lipid peroxidation (malondialdehyde) was determined in six areas of the brains of Alzheimer's disease and control subjects, matched for age (74 ± 7 and 73 ± 8 years, respectively) and postmortem delay (10 ± 6 and 11 ± 7 hours, respectively). Malondialdehyde (MDA) concentration was significantly increased in all samples. Basal and iron/orotate-stimulated malondialdehyde concentration were 19% higher in the inferior temporal cortex of Alzheimer's disease subjects than in control subjects; other regions were unaffected. In order to assess antioxidant capacity, we determined the activities of glutathione peroxidase and glutathione reductase in addition to two enzymes of the peroxynitrite pathway (glutathione peroxidase and 6-aminohexanoylglutathione dehydrogenase) and two enzymes of the nicotinamide adenine dinucleotide (NADH)-dependent pathway (glutathione reductase and 6-aminohexanoylglutathione dehydrogenase) in three areas of neocortex. The only difference was an increase in the activities of glutathione peroxidase and glutathione reductase (to 126% of control and to 126% of control, respectively) in the inferior temporal cortex of Alzheimer's subjects. Together these data indicate that lipid peroxidation is increased in Alzheimer's disease because of increased prooxidant activity rather than diminished antioxidant defenses. Moreover, it appears that the activity of pentose phosphate pathway is increased in response to increased prooxidant activity since 6-phosphogluconate dehydrogenase activity (0.89, p < 0.01) with the stimulation of MDA. This inverse relationship suggests that elevations in the activity of pentose phosphate oxynitrogen reflect a compensatory change in response to increased prooxidant activity. Although it is not clear if increased prooxidant activity is a cause or a consequence of early pathological change, it is consistent with the involvement of oxidative stress in the maintenance and progression of the cascade. Antioxidant therapy can therefore be expected to stop, or at least slow, the progress of this debilitating disease of late life.

522.6

GIF, or metallophilic-3 (MT-3), a member of the metallophilic family, is located in the molecular layer of the dentate gyrus, the pyramidal cell layer of the hippocampus, and layers 2-6 of the neocortex. GIF is expressed in astrocytes but is down-regulated during fetal development and in AD brain.

Western blots for GIF, GFAP, and total metallophilic-3 (MT-1; MT-2 and GIF; MT-3) were performed to quantify these proteins in temporal lobe of control and AD brain. Levels of MT-3, in control and AD temporal cortex were indistinguishable. However, GIF levels were unchanged when compared with control. Conversely, there was an 83.4% increase of GIF expression in AD brain when compared with control. Immunocytochemical detection of the same proteins was performed on sections of control and AD from the middle temporal gyrus. Double immunostaining suggested that GIF is down-regulated in the cytoplasm, nucleus and processes of astrocytes even though reactive gliosis was found in AD brains. This was reflected in heavy GFAP staining of astrocytes in AD sections. Furthermore, even though no differences in MT-1, expression were apparent when assessed by blotting, immunostaining suggested a marked increase in MT-3 expression and a loss of GFAP staining in glial cells. This suggests that GIF is an important protein found to be down-regulated during gliosis in AD.

522.8
SELECTIVE ABNORMALITIES IN MULTIPLE NEUROTRANSMITTER RECEPTOR DENSITIES IN NEURODEGENERATIVE DISEASES. L. R. Murtha*. VA Med Ctr./Mt Sinai Sch of Med./NYC, NY.

This study investigates the densities of multiple neurotransmitter Receptors, in ten different cortical regions of human brain, obtained from patients: Normal, Schizophrenia(Sch), Alzheimer's disease(AD) and Multi Infarct Dementia(MID).

Aliquots of membrane suspension obtained from each cortical region were analyzed simultaneously for the binding of radiolabeled nicotine, ketanserin and SCH 23390 to nicotine,serotonin 5HT2 and dopamine D1 receptors respectively. The receptor binding assays were done in the absence and presence of cold ligands to obtain specific and non-specific binding respectively of radiolabeled ligands. Important differences in the densities of the three different neurotransmitter receptors were noted in the ten cortical regions of Sch, AD, MID brains as compared to normal-demented elderly. 

522.10
NEUROTOXIC EXTRACTS OF ALZHEIMER'S DISEASE BRAIN ARE ASSOCIATED WITH REACTIVE MICROGLIA. L.J. Havercamp* and J.B. Kirkpatrick. Dept. of Neurology and Pathology, Baylor College of Medicine, Houston, TX 77030.

We have adopted a triple stain for plaques (thioflavine-S), cell nuclei (bismacinid), and reactive microglia (immunochemistry for HLA-DR) in tissue obtained after relatively short post-mortem intervals (< 6 h), lightly fixed in parformaldehyde. Using this technique, we note as especially prominent in Alzheimer's disease (AD), clusters of three or more reactive microglia, invariably in association with all (99%) core smalle plaques, a majority (40-85%) of neuritic plaques, and none of the diffuse plaques which are often seen in brains (matched for age and post-mortem interval) lacked toxic effect. Toxic activity was regionally distributed within AD brains (N = 7), with hippocampus invariably containing high levels of toxicity while cerebellum and white matter had little. Correlations of histopathology and toxicity of adjacent tissue, from 8 regions in each of the control and AD brains, showed a close relationship between neuron-killing activity and the density of microglial clusters associated with core smalle toxic and neuritic diffuse plaques. These findings suggest participation of activated microglia-derived neuronal toxins in the neuron death which occurs in AD.
522.3

Our hypothesis is that cell patterns in the neural tube are controlled by a coordinate system of positional information, set up along the antero-posterior (AP) and dorso-ventral (DV) axes, and that the AP axis of the chick hindbrain becomes subdivided into a series of compartments (rhombomeres) that underlie its segmented architecture. Coincident with the emergence of territories where cell mixing is transiently restricted, the rhombomeres become determined for expression of a specific combination of selector genes (Hox genes) that may encode rhombomere identity. Rhombomere phenotype is determined at this stage. Thus, when presumptive rhombomere 4 (r4) is transposed for r2 (at 6 somites), the graft expresses a r4-specific gene (Hoxb-1) and develops r4-specific neurons. We now find that only the AP and not the DV positional value of the rhombomere is determined at this time. Thus, when r4 is grafted to the r2 position with its DV polarity inverted (the alar plate positioned ventrally, abutting the floor plate) r4-specific motor neurons appear medially, within the original alar plate. Cells are committed to their fates according to position on a Cartesian grid whose coordinates are set sequentially; DV positional values are labile when AP values are already fixed.

522.4

Comparative phylogenetic models have great potential for establishing how gene expression confers positional identity to cells, an identity that in turn largely predisposes early embryonic neural pathways and innervation patterns. Despite the diversity of the adult brainstem organization, recent data suggest that the overall segmental, rhombomeric (Rb) blueprint of the hindbrain has been genetically conserved in vertebrates. The phylogenetic diversification of cellular identity related to derived sensory-motor circuits responsible for vertebrate posture can now be addressed within this Rb framework. Dye labeling demonstrates that cranial and spinal somatosensory neurons arise as largely non-overlapping segmental populations arising rostrally to caudally along the Rb neural axis with homotopic subdivision of cranial nerves occurring in different species. Surprisingly, the hindbrain areas identified as different to eye, head and spinal sensory-motor circuits are ordered posteriorly to anterior in the module of diverse vertebrates. In particular, we have documented the morphology and physiology of individual, non-overlapping nuclei extending through regions arising from Rb 8 to 4 in tetrapods. Notably, the two most evolutionarily derived hindbrain centers are known as lateral and ventrolateral temperature, are located just rostral to the spinal cord and are presumed to originate from Rb 8. Two other general classes of nuclei are located further rostrally, likely arising from Rb 7. Comparison of homologous posterior circuits between species, even when given embryonic hindbrain compartment must coordinate the developmental plans of diverse neuronal types that are acquired and combined through stepwise phylogenetic elaboration. We propose that the need to insert novel, derived circuits (e.g., the integrations) into established, more primitive, hindbrain sensory-motor blueprints constrains the functional organization of downstream targets of homologous sensory genes. As a result, gene deletion/segmental expression experiments involving high level selective genes can be expected to produce a mosaic pattern of variation arising both ancient and recently acquired species-specific neural circuitry. Our hypothesis that to co-dependently address the molecular correlates of neuronal development, experimental analyses will require consideration of the phylogenetic diversity of neuronal structure and function observed in embryonic rhombomeres.
$\text{52.3.1} $ 

**CELL LINEAGE IN THE FORMATION AND REGENERATION OF THE OLFATORY PLACODES.** C.D. Burau*, D. Collazo, and S.E. Fraser. Dept. of Molecular and Cellular Biology, Univ. of Arizona, Tucson, AZ 85721 and Div. of Biology, California Institute of Technology, Pasadena, CA 91125.

Previous studies in Xenopus demonstrated that the olfactory placodes are derived from the neural plate (Eagleson and Harris, 1990). We and other laboratories have also observed that the olfactory placodes can regenerate (Byrd and Burd, 1993). The goals of the current study were to demonstrate the origin of the olfactory receptor neurons during normal development and to determine the origin of olfactory receptor neurons in regenerating olfactory placodes. The lineage of cells was determined by labeling small groups of cells with DiI/SCy3 or single cells with lysinated rhodamine dextran (NRD), which was allowed to follow cells using low light level video microscopy. Cells in the anterior-lateral region of the anterior neural ridge (labeled at stages 14-16) give rise to olfactory placodes and form the olfactory and vomeronasal epithelia in tadpoles (stage 48). At later stages (stages 22-24), cells in the same plate persist to and during olfactory placode formation give rise to olfactory receptor neurons and supporting cells. Following removal of the olfactory placodes (stages 33-34), we found that undifferentiated cells surrounding the olfactory placodes in the same plate give rise to new olfactory placodes and give rise to new olfactory receptor neurons. In summary, undifferentiated cells in the same plate retain the potential to form olfactory receptor neurons well after olfactory placodes have formed. The molecular signals and genes involved in this process remain an exciting avenue for future investigation. Support: NSF, MDA, and NIMH.

$\text{52.3.7} $ 

**NOTCH IS A DETERMINANT OF RETINAL GANGLION CELL FATE IN THE CHICK.** C.P. Austin*, D. Feldman, J. Ma, S. Field, Barry, and C.L. Cepko. Dept. of Genetics, Harvard Medical School, Boston, MA 02115.

The factors affecting cell type determination and differentiation in the vertebrate retina are not well understood. In the chick, ganglion cells are the first cell type to be produced in the chicken retina, and we have used an in vitro system to study the factors influencing their development. The presence of an inhibitor of GC differentiation that is provided by the neighboring retinal pigment epithelium (RPE) is required for ganglion cell development at low density; below a critical cell number, the ganglion cells are dissociated and cultured at low density, the percentage of cells expressing GC-specific markers increases progressively (NIMH, CSH). (Abstr. 519:13.15.93)

The Notch gene family has been hypothesized to code for cell surface receptors that mediate inhibitory signals influencing cell fate in a number of organisms. We have observed that Notch plays a role in the chick retina, as an inhibitor of GC differentiation. When antisense oligonucleotides directed against any of 3 regions of the Notch sequence are added to retinal explant cultures, the number of GCs is increased by at least 25% over 24 hours relative to explants incubated with sense oligo controls. To test the specificity of the oligo sequence in bringing about GC overproduction, we have used antisense oligonucleotides mismatched at 1, 3, or 5 bases, and found that the extent of GC overproduction decreases with increasing mismatch. Western blots showed that Notch protein level is greatly diminished in mutant lines treated with antisense oligonucleotides, relative to sense oligonucleotide, or non-oligo-treated controls. Expression of a truncated form of the human Notch homolog (TJN), corresponding to the intracellular portion of the protein, via retroviral transduction reduced the number of GC produced in vivo and prevented the overproduction of GC previously seen in dissociated culture. These findings support that Notch is an inhibitor of ganglion cell determination in the early chick retina, and supports the emerging hypothesis that the GC gene family plays a central role in neuronal determination in vertebrates. Supported by K11 HE 00231 (CPA) and R01 HE 00767 (CLC) from the National Eye Institute.

$\text{52.3.9} $ 

**ORIGIN OF SUBVENTRICULAR ZONE CELLS.** M.C. Mione* and J. G. Papavassiliou, Department of Anatomy, University College London, Gower Street, London WC1E 6BT, U.K.

In the late stages of cortical neurogenesis, the subventricular zone (SVZ) is a prominent layer containing numerous mitotically active cells. It is believed that these cells give rise to the astrocytes and oligodendrocytes of the cerebral cortex and the subcortical white matter. In order to investigate whether SVZ cells originate from ventricular and subventricular (SVZ) cells, we made retroviral transneuronal injections of a recombinant retrovirus carrying the reporter gene for E. Coli β-galactosidase at different embryonic ages. Rats were killed after 3 days in utero or 3 days after birth at 2, 4, and 6 days (P) 3-4. All animals, irrespective of the age of injection, showed β-gal+ SVZ cells when examined at birth or later. Therefore, we concluded that cells present in the VZ between E15 and E19 gives progeny to E15 or even earlier. If all E15 SVZ, β-gal+ cells were present in the SVZ at least until P14, although their number was greatly reduced by this age. In order to ascertain whether SVZ cells originate from a population of neuroepithelial cells or if they are stem cells left behind by their migrating progeny, we used bromodeoxyuridine (BrdU) to distinguish between mitotically active or quiescent cells. BrdU was given every two hours over a period of 24 hours starting 2 days after retroviral injection. Animals were killed shortly after the last injection or at birth. It was found that the majority of β-gals in the SVZ was BrdU, thus indicating that they were mitotically active during the period of BrdU injection. This finding suggests that stem cells in the VZ give rise to the glial present in the SVZ. Supported by the Wellcome Trust.

$\text{52.3.10} $ 


Using two-dimensional gel electrophoresis we previously identified a 46 KDa protein (protein 310) that is neural specific, abundantly expressed in the embryonic cerebral cortex, and down-regulated in the adult (J. Neurosci. 9:304). Antibodies to synthetic peptide fragments from protein 310 initially induced rat embryonic cerebral cortical neurons immunohistochemically distinct from endogenous ERG-Rs. The vector also contains an IRES sequence, which allows a second encoding of the rat β-gal to be expressed at high levels in infected cells. For example, 98% of retinal cells that express virally transduced β-gal also express β-Gal. The co-expression of β-gal facilitates analysis of the proliferation, survival, and differentiation of infected cells. Expression of β-gal in rat retina infected with virus expressing ERG-Rs and β-gal, or with virus expressing β-gal alone. Proliferation (measured by BrdU incorporation) was analyzed 6-7 days after infection as the expression of β-gal and injection of BrdU by quin expression was analyzed 11-16 days after infection, using rabbit anti-β-gal together with mouse anti-BrdU or mouse anti-opi (Rn-91) antibodies. Proliferation of retinal cells infected with β-gal-RV was enhanced by 30-300% in response to endogenous ligand (no TGFs added) or exogenous TGFs (0.1 ng/ml). Development of retinal cells infected with β-gal-RV was induced at 7 days and could be blocked by quin expression. These observations suggest that limits of specific expression contribute to the regulation of proliferation and differentiation in the retina, and that these restrictions can be overcome by manipulation of levels receptor expression.

In vivo, neurogenesis in the olfactory epithelium (OE) proceeds continually, but in vitro, in minimally supplemented defined medium, Immediate Neurogenic Precursors (IPs) of olfactory receptor neurons (ORNs) divide only once. We found that Fibroblast Growth Factors (FGFs) and transforming growth of IPs of orf cultures from E14-15 mouse embryos, while other polyepthelial growth factors tested did not.Expression of FGF receptors, detected by RT-PCR in RNA isolated from E14-15 OE, suggests that FGFs may stimulate FGF receptors in vivo as well. Labeling INPs through successive 5 with bromodeoxyuridine (BrDU) and 3H-thymidine (3H-TDR) demonstrated that INPs allow a single cell to undergo symmetric division by differentiating into ORNs. Varying the interval between BrDU and 3H-TDR pulses allowed length of the INP cell cycle to be estimated at 17 hr with a phase of 8 hr; this finding enabled us to estimate that FGF exerts its effects in late G2M or early G1. Neurogenesis usually ceases by 48 hr in culture, and in OE, because >90% of IP progeny differentiate into postmitotic ORNs. However, in rare events growing in FGF, large groups of proliferating INPs are seen after this time, suggesting that a progenitor cell--possibly a stem cell--is giving rise to new INPs in these explants. These experiments suggest that the transcription factor Mammalian Auracine Scale Homolog 1 (MASH1) may be required at an early stage of genesis of ORNs (Guillemot et al., Cell 75:463-476 [1993]). We find that a subpopulation of proliferating migratory cells in early (16-20 hr) OE cultures expresses MASH1, but expression declines rapidly; this decline is not due to cell death. MASH1+ cells are immunologically and morphologically distinct from basal cells, and their high β-Gal-activity index suggests that they are not stem cells. The data are consistent with a model of neurogenic neurogenesis in which the MASH1-expressing cell is an early stage of, or progenitor to the INP, which as a neuronal transit amplifying cell.
ELIMINATION AND DETERMINATION III

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Neuronal csk-5 phosphorylates the high molecular weight neurofilament proteins (NF-H). During the purification of this kinase, we identified a molecule of 67 kDa (P67) which is associated with and activates csk-5 (Shute, et al, 1990) Abs. Soc. Neurosci. 16:193. A molecule, called MK-801, identical to P67, was shown to be associated with synapsin-I, one of the key molecules of the synaptic vesicle fusion protein complex (Matsudaira et al, 1993, Nature 366:347). To further explore the function of MK-18, either as a regulator of csk-5, or as a component of the synaptic vesicle complex, (or both), we investigated the immunohistochemical expression of csk-5, MK-18, synapsin and NF-H during the development of the rat cerebellum from P2 to the adult. We assumed that if these molecules were functionally related, then they should (1) co-localize within the same cells and tissues, and (2) undergo similar patterns of developmental regulation. Using antibodies to csk-5, NF-H, synapsin and MK-18, we could show that at all stages, the epitope shared by MK-18, synapsin and MK-18 were co-localized into developing molecular layer (parallel fibers), the synaptic glomeruli in the inner granule cell layer, and in afferent and efferent fibers (climbing fibers, mossy fiber and Purkinje cell axon) within each folium. Only csk-5 and MK-18 antigens were detected within the Purkinje cell bodies. The pattern of NF-H expression, however, showed differences since it was absent from the molecular layer through most of development, but was primarily restricted to axons and fibers bundles within each folium. At late developmental stages, NF-H immunostaining was seen as dendiric and surrounding some regions of Purkinje cells. The expression patterns of csk-5 and MK-18 during development, as seen immunohistochemically and in immunoblots, are similar, which is consistent with the hypothesis that they are functionally related.

552.1 EVIDENCE FOR A ROLE OF STRIATAL GABA IN THE ANTAGONISTIC DOPAMINE - GLUTAMATE REGULATION OF LOCOMOTION: AN IN VIVO MICRODIALYSIS STUDY IN THE AWAKE FREELY MOVING RAT. W.T. O'Connor*, M. Morari, U.Ungerstedt and K. Fuxe, Departments of Pharmacology and Neuroscience, Karolinska Institute, Stockholm, Sweden.

The effect of kainic acid-inducedornithine (TTX) in the substantia nigra pars reticulata (SNr) on behavior and basal dopamine (DA), GABA, and glutamate (GLU) levels in the dorsolateral striatum was monitored using in vivo microdialysis in the awake rat. Basal striatal DA, GABA and GLU levels (nM) were 1.7 ± 0.9, 10.4 ± 0.7 and 307 ± 40 respectively. Nigral perfusion (70min) with TTX (10μM) produced a strong contralateral rotation (8.5 ± 4.4 min after 30 min); a decrease in ipsilateral striatal DA release (-76% of control) and an increase in striatal GABA (+46%) and GLU (+80%) release. Intrastriatal perfusion with MK-801 (10μM) alone did not affect behavior or striatal neurotransmitter release but counteracted the contralateral rotation (1.3 ± 0.5 min after 30 min); abolished the increase in striatal GABA release and delayed the increase in striatal GLU release associated with perfusion with TTX in the ipsilateral SNr. The reduction in striatal DA release associated with nigral TTX was not affected by intrastriatal MK-801. Thus, the counteraction of the nigral TTX induced increase in both rotational behavior and striatal GABA release following intrastriatal perfusion with MK-801 strengthens striatal GABA neurons as a major target for the antagonistic DA-Glu interaction in the regulation of locomotion.

552.4 SEIZURE PROTECTION WITH IB-MECA, A SELECTIVE AGONIST OF THE NOVEL ADENOSINE A3 RECEPTOR. D.K.J.E. von Lubitz*, M.C.Carter†, R.C.S. Lish†, K.A. Jacobson*, NINDS/NIH/Molecular Recognition Section, Bethesda, MD 20892; 2Dept. of Physiology and Biophysics, Hahnemann University, Philadelphia, PA, 19102.

The biological function of adenosine A3 receptors is virtually unknown. We therefore, investigated the effect of acute and chronic preischemic stimulation of these receptors on the outcome of NDMA or pentyleneetetrazol (PT) induced seizures in C57Bl/6J mice (N=10/group). P. with N6-(3-iodobenzyl)-adenosine-5'-N-methylcarboxamidazole [IB-MECA (10, 50, 100 μg/kg)] 15 min prior to either NDMA (60 or 125 mg/kg) or PT (75 mg/kg). In the chronic regimen NDMA (60 and 125 mg/kg) was significantly delayed by acute and chronic IB-MECA at doses >50 μg/kg. At 100 μg/kg both acute and chronic IB-MECA significantly reduced postictal mortality in both NDMA and PT groups. In a second experiment of 120 μg/kg IB-MECA improved survival of PT induced seizures even further. Our results indicate that, contrary to its effect in cerebral ischemia, acutely injected IB-MECA is highly protective against seizures elicited by different epilepticogenic drug classes and that, as in stroke, chronic treatment with IB-MECA offers an equally significant seizure protection. In view of the therapeutic value of adenosine A3 receptor stimulation is highly warranted.

The amine agmatine (Agm) (decarboxylated arginine), is an endogenous ligand for imidazole (I) and D-glutaminergic receptors present, with its biosynthetic enzyme ADC, in bovine and rat brain medulla. While prevalent in bacteria, neither Agm nor ADC have previously been found in mammalian brain. We investigated whether mammalian ADC has unique properties. ADC was assayed by measuring conversion of [5-3H]Arginine to [5-3H]Agmatine in bovine and rat brain medulla. While previous studies have shown the existence of Agm in bovine and rat brain medulla, these studies were performed using the agmatine detected by measuring the amount of Agm produced after incubation with [5-3H]Arginine. Our results confirm the presence of Agm and show that ADC is present in both bovine and rat brain medulla. These findings indicate that Agm interacts with receptors close to its site of biosynthesis.

524.7 SITE-DIRECTED MUTAGENESIS OF THE HISTAMINE H1-RECEPTOR INDICATES A SELECTIVE INTERACTION OF Astm. WITH SUBCLASSES OF H1-RECEPTOR AGONISTS. R. Worden, D. M. Smith, C. P. Fraser, A. M. TerLeak and H. Timmerman, Leiden/Amsterdam Centre for Drug Research, Department of Pharmacoochemistry and Graduate School for Neurosciences, Department of Biochemistry and Molecular Biology, Vrije Universiteit, 1081 HV Amsterdam, The Netherlands.

The cloning of gene encoding the H1 receptor made it possible to study the interaction of subtype specific ligands with the receptor protein. In this study we investigated the role of the Astm. and the Thr210 residues in TMS of the guinea-pig histamine H1-receptor by site-directed mutagenesis to non-functional alanines. After stable expression of the receptor mutants in Chinese Hamster Ovary cells the pharmacological properties of the receptor proteins were investigated by [3H]mepyramine binding studies and [3H]histamine phosphate accumulation. Whereas the Thr210 is not important for the action of histamine, the Astm. residue appears to be involved in the binding of the N3-nitrogen atom of histamine and its 2-methyl analogue. For the (2-3-bromophenyl)-alanine and the non-imidazole H1-receptor agonists 2-pyridyl ethylamine and 2-thiazolidyl ethylamine the Astm. is not essential for binding. On the basis of this study we conclude that different histamine H1-receptor agonists interact in different ways with the receptor proteins. Moreover, we speculate that the interaction with the N3-nitrogen atom is essential for receptor activation.


The screening of a guinea pig histamine H2 receptor cDNA library with probes derived from the sequence of the rat histamine H2 receptor, allowed us to isolate an intronless gene encoding a protein of 359 amino acids displaying all major features of G-protein-coupled receptors and a 94% homology with the rat histamine H2 receptor. Northern blot analysis, performed with a specific C-terminal tail DNA probe derived from the guinea pig sequence revealed a 4.6 kb transcript in various guinea pig tissues. In brain, a high expression was found in striatum, brainstem, olfactory tubercles and bulb. A signal was also present in hippocampus, cerebellum, hypothalamus and substantia nigra. In peripheral tissues, the expression was detected in stomach, lung and heart, where the labeling was very high in ventricles but hardly detectable in atrium. In situ hybridization studies showed that highly expressed guinea pig cerebral and cerebellar expression of the H2 receptor gene transcripts which was compared to a detailed autoradiographic mapping of the receptor using 125I- imidodinopentetide. There was a spatially sharp correlation between the distribution of the two markers. A high labeling was detected in striatum, Calleja islands, olfactory tubercles, cortex, hippocampus, pontine nuclei, inferior olive. Amygdaloid complex, lateral geniculate nucleus, superior colliculi and median thalamus were also labeled. An interesting discrepancy exists, however, in the hippocampus complex where the binding sites were high in the molecular layer, whereas the mRNA was localized in the pyramidal or granular cell layers. Finally, using a chromosome mapping panel constructed from somatic cell hybrids, we assigned the human histamine H2 receptor gene to chromosome 5 showing that the genes encoding the H2- and the H2-receptor are not clustered.


It has previously been shown that diphenhydramine hydrochloride (Benadryl) eliminates or reduces symptoms of dystonia in human patients with acute dystonic reactions associated with procyclidine treatment. This study of the effects of diphenhydramine in the treatment of idiopathic torsion dystonia are related to the H1-selective antihistaminergic actions of the drug. In addition, the H1 agonist pyrilamine and the H2 antagonist cimetidine are not effectively reducing dystonia. The results also provide support to previous research which has implicated the red nucleus in the pathophysiology of dystonia. The results of drugs injected into the red nucleus produced an observed by measuring the angle of deviation of the head from the dorsal plane. A significant rotation of the head is indicative of dystonia. Histamine (dose range 0-10 nmol) produced dose dependent dystonia in rats (P<0.001). The dystonia produced by a dose of histamine (10 nmol) could be antagonized by coadministration of diphenhydramine (P<0.01), pyrilamine (P<0.05), and cimetidine (P<0.04). Significantly more dystonia was observed when histamine was injected into the red nucleus rather than midbrain area surrounding the structure (P<0.002). These results indicate that the beneficial effects of diphenhydramine, pyrilamine, and cimetidine in reducing symptoms of idiopathic torsion dystonia occur via a histaminergic pathway involving the red nucleus.


Recent studies have shown the existence of a specific antagonistic interaction between adenosine A1 receptors and dopamine D2 receptors in the brain. This A1/D2 interaction seems to be relevant for the behavioural effects of adenosine agonists and antagonists, like caffeine. In the present study quantitative receptor autoradiography and brain microdialysis were combined to demonstrate a powerful antagonistic A1/D2 interaction in the ventral striopallidal system. In the presence of the A1 agonist CGS 21860, dopamine exhibited a lower affinity in displacing the labelled D2 antagonist by infusing into the accumbens but not by infusing into the substantia nigra. The results also show that dopamine uptake into the accumbens is selective. However, the current in the nucleus accumbens was reduced by coadministering CGS 21860 with caffeine, which is a potent competitive A1 receptor antagonist. These findings are consistent with the idea that the combination of CGS 21860 and caffeine could be used to ameliorate the effects of adenosine antagonists, like caffeine, and to potentiate the effects of dopamine agonists, like caffeine, in the treatment of schizophrenia.

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$24.11$


We have demonstrated synergistic interactions between adenosine-2 (A-2) and dopamine-2 (D-2) receptors in a behavioral rat model. Because A-2 and D-2 receptors are co-localized on the same type of striatal neurons, we studied the effects of chronic neuroleptic administration (haloperidol) on A-2 and D-2 receptors in rat brain. Rats received 21 daily injections (i.p.) of haloperidol (1.5 mg/kg), fluphenazine (1.5 mg/kg), sulpiride (100 mg/kg), clozapine (20 mg/kg), or saline. Striatal receptor density (Bmax) and affinity (Kd) values for A-2 and D-2 receptors were measured using a high-affinity radioligand. Tryptophan hydroxylase-labeled D2 receptors were measured using homogenate binding. Haloperidol and fluphenazine significantly increased (>30%) striatal A-2 and D-2 receptor density, whereas sulpiride and clozapine had no significant effect. Kd values for [3H]CGS25380 were unchanged in all groups. Thus, these observed significant increases in the density of A-2 receptors in rats striatum following chronic administration of typical and atypical striatal A-2 receptors, whereas sulpiride and clozapine was not effective.

$24.12$

**STIMULATORY EFFECTS OF SUB-ANEUPHLETIC DOSES OF KETAMINE. A PSYCHOTOMIMIC NMDA RECEPTOR ANTAGONIST?**


Sub-aneuphletic doses of ketamine, a non-competitive NMDA-receptor antagonist, cause several schizophrenia-like symptoms in non-human primates including impaired performance on the Wisconsin Card Sorting Test (Kray et al., Arch. Gen. Psychiat. 1994). In this study, intracerebral microdialysis in conscious rats was used to assess the effect of ketamine on the extracellular levels of dopamine and glutamate in the prefrontal cortex. A sub-aneuphletic dose of ketamine (10, 20, and 30 mg/kg, i.p.) increased glutamate levels while the dopamine levels were largely unaffected at these levels. An intermediate dose of 50 mg/kg was without an effect. This finding is similar to our previous data with other types of NMDA receptor antagonists. A recent study indicates that ketamine may exert some of its effect by increasing the levels of endogenous excitatory amino acids, thus, causing a stimulation of postsynaptic (non-NMDA) excitatory amino acid receptors. Supported by MH48404, MH48486, VHVA Center for Schizophrenia and VA Merit Award.

**EXTRASTRIATE VISUAL CORTEX: PARietAL AREAS II**

$25.1$

**EFFECTS OF VESTIBULAR AND NECK PROPRIOCEPTIVE SIGNALS ON VISUAL RESPONSES IN POSTERIOR PARIETAL CORTEX.** H. L. Snyder*, S. A. Anderson. Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093.

Eye, head and body position modulate the discharge of retinotopically tuned units in posterior parietal cortex (PPC). The conjunct of retinal and extraretinal signals give coordinate transformations from visual to body- or world-centered reference frames. We investigated the sensory modalities responsible for extraretinal signals in areas 7a and 7p of macaque monkeys. Vestibular signals modulated effects of body position in space on single units in PPC. Animals were seated on a vestibular turntable in a light-proof room. Saccades to retinotopically identical locations were elicited after whole-body displacement in the dark. The discharge of single units was influenced following rapid (10/15s) or gradual (0.04/10s) rotations (7/12 cells), ruling out uncoordinated body or visual or other signals to body position in space.

Neck proprioceptive signals mediated effects of head-on-body position. Proprionicvestibular, visual and visual signals may all be affected by head displacement. To show that changes in proprionicvestibular signals alone were sufficient, whole-body rotation in the light was followed by counter-rotation of the head back to the starting position in the dark, resulting in body but not head displacement. This resulted in saccadic saccades subterminally elicited in the dark revealed single unit modulation identical to that obtained by merely rotating the head on a stationary body (11 units).

Vision influenced the effect of position in space on single units. Following whole body rotation in the light, responses to identical saccades elicited in the dark were often modulated by body position (8/22 units). In contrast, body position was much less likely to effect saccadic responses following whole body rotation in the dark (7/58 units). Our results suggest that in the absence of vision, the PPC boosts its reliance on vestibular signals.

$25.3$

**EGOCENTRIC MOTION FROM OPTIC FLOW AND EYE POSITION IN AREA 7a OF THE BEHAVING MACAQUE.** R.M. Segraves and J.L. Ilg*, Dept. of Neurobiology and Anatomy, Center for Molecular and Behavioral Neuroscience, Rutgers University, New Brunswick, NJ 08902.

Eye position signals were found to be correlated with direction heading in head-centered coordinates. Recordings were made from area 7a while the monkey was performing a reaction time visual pursuit task. The monkey did motion stimulus elicited perceived direction of gaze. Direction was estimated using a two-dimensional vector whose components were the projection onto the horizontal and vertical axes of the vector representing the direction of gaze. Perceived direction was defined as the projection of the vector representing the movement of the eye from one position to another onto the same central retinotopic area.

Area 7a neurons were found with translation torque, and planar rotational selectivity or receptive field (RF) movement in area 7a. For example single units could be selective for both clockwise rotation and radial expansion. This suggested that there might be cells selective to spiral motion was produced earlier on psychophysical grounds (Anderson and Siegel, 1995). Neurons were found with selectivity to clockwise rotation. These neurons seemed to be a severe coarsening for the type of structured motion in area 7a which could be principle used to be determined.

The dependence of the response of these optic flow selective neurons in the retinal focal of the monkey was what and when RFs were similar to the RFs of macaque area 7a. For example, single units could be selective for both clockwise rotation and radial expansion. This suggested that there might be cells selective to spiral motion was produced earlier on psychophysical grounds (Anderson and Siegel, 1995). Neurons were found with selectivity to clockwise rotation. These neurons seemed to be a severe coarsening for the type of structured motion in area 7a which could be principle used to be determined.

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525.5

OPTIC FLOW RESPONSES OF MST NEURONS DURING PURSUIT EYE MOVEMENTS

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The retinal image of an optic flow field is altered by concurrent smooth pursuit eye movement. We consider the task of interpreting that image, information about pursuit, if available to the visual system, might simplify that interpretation. We recorded the responses of 117 MST neurons to optic flow stimuli, during smooth pursuit, and attempted to determine whether the response of these cells is altered by smooth pursuit.

We recorded from 117 single neurons in area MST in two monkeys. We first determined (rarely with optical or circular) evoked the strongest response when the center of motion (COM) was centered on the screen. We then presented the preferred pattern, with 8 different created by combining 8 directions of planar motion, shifting the COM to 8 different positions in the stimulus. Ninety-four percent of the neurons responded differently to the centered and shifted COMs. Each neuron was then tested with the centered COM during smooth pursuit in 8 different directions. Eight-five percent of the neurons showed different responses to different pursuit directions. We compared the shifted COM response with those to pursuit across the centered COM, and found that most neurons (61%) had the same response to the retinal image, whether it was created by optic flow, or optic flow with pursuit. However, 39% responded differently when pursuit effects were contributed to the retinal image. In addition, over half of the neurons (56%) responded during smooth pursuit across a blank screen. These studies suggest that some MST neurons access both optic flow and smooth pursuit signals, potentially contributing to optic flow analysis during smooth pursuit eye movements.

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525.6

RESPONSES OF LIP NEURONS DURING A MOTION DISCRIMINATION TASK: A DECISION PROCESS IN ACTION

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Visually guided behavior requires selection or decision processes that identify one among many possible objects as the target of a movement. We investigated one such decision mechanism in a rhesus monkey trained to discriminate the direction of movement in a visual scene to indicate its direction by executing a saccade to one of two possible targets. During performance of the task, we recorded from neurons in a parietal area that receives projections from MST and MT and participates in the planning of saccades.

In each experiment, we placed one potential saccade target within, and the other outside, the movement field of the neuron under study. Each trial contained a 2.2 second stimulus presentation and a delay period prior to the saccade. Not surprisingly, delay period activity of LIP neurons reliably predicted the monkey’s eye movement: activity was stronger on trials in which the monkey chose the direction of motion toward the movement field. More interestingly, LIP neurons also signaled the monkey’s impending choice during the stimulus presentation interval, well before the saccade. This predictive activity built up earlier and was more reliable for easy than for hard discriminations (strong vs. weak motion signals). In other words, LIP neurons appeared to accumulate evidence regarding the direction of motion in the stimulus and to maintain a response suitable for guiding the appropriate saccade. Little evidence for this kind of activity was observed in control blocks of trials in which the same stimulus was presented, but the animal was not required to perform the direction discrimination. Thus the activity of LIP neurons may reflect an active decision process, but in which sensory signals are evaluated to inform target selection. Supported by NSF (87-04560), training grant NS 07158-14, and HHMI.

525.7

RELIABLE TEMPORAL MODULATION IN CORTICAL SPIKE TRAINS IN AWAKE MONKEY

W. Bartel1, C. Koch,1 R. C. Olshausen1,2, K. Britten3, and R. Grossman1.

1Department of Neurobiology Stanford, Stanford, CA 94305; 2Center for Neuroscience, UC Davis, CA 95616.

We analyzed the repeatability and precision of temporal structure in spike trains recorded from area MT in behaving rhesus monkeys which viewed a dynamic random dot stimulus that did not vary from trial to trial (see data; Olshausen, Britten, and Mowshon, 1989). We find a surprising degree of regularity in the temporal modulation of the response of many cells—the most reliable cells will fire a few action potentials or even a period of elevated firing which begins and ends fixed in time relative to stimulus onset with a standard deviation of less than 4 msec in recording periods which may last many minutes to hours. Spike count during these short periods of elevated firing rate is more reliable, i.e. has a lower variance to mean ratio, on average than the spike count taken over the entire 2 sec stimulus. Offset and high of elevated firing periods can occur with very similar and fast time constants. Autocorrelation analysis reveals that deviations from the mean response are rarely substantially correlated beyond 100 msec, and in half of cells no correlation exists between consecutive interspike intervals beyond that predicted from the time-varying mean firing rate. Reliable temporal modulation disappears or diminishes for completely different motion stimuli, and we propose an experiment to determine whether this is due to saturation in firing rate or due to a qualitative change in the way MT cells respond to the stimulus.

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525.8

INTEGRATION OF MOTION AND STEREO CUES IN MACAQUE VISUAL AREA MT

D. C. Bradley and R. A. Andersen, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Most neurons in primate medial temporal cortex (MT) are highly sensitive to motion in a visual field. Recently, many MT neurons have been recorded from areas of the posterior parietal cortex, i.e., areas adjacent to MT. Simultaneously, a number of studies have shown that covert movements evoked by a stimulus presented in the opposite visual field are maintained for periods of time, i.e., the so-called "opposing" movement occurs simultaneously in a receptive field. An important benefit of this "motion opponent" is that random motion signals created by non-motion stimuli (e.g., flicker) tend to cancel out. However, motion opponent may also be impaired to MT responses under conditions of temporal motion, where motion signals are often locally opposed and as such tend to negate each other in MT. We hypothesized that this pattern might be overcome if opponent signals from different visual depths (and thus different layers) did not occur. To test this idea, we stimulated 146 MT neurons with a random dot pattern moving in the preferred direction and at the normal disparity (i.e., depth) for a given cell. A second pattern moving in the antipreferred direction was introduced, and the suppression due to this pattern was measured as a function of disparity. In ~40% of the cells, suppression was greatest when the antipreferred disparity was similar to the disparity of the preferred pattern and comparable to the normal disparity (as defined for preferred motion) regardless of the actual disparity of the preferred pattern. Our findings thus suggest that motion opponency in MT occurs mainly within a given disparity channel. This disparity-specific opponency favors the cancellation of random motion signals from a given depth, while allowing independent representation of surface movements at different depths.

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525.9

SINGLE NEURONS IN POSTERIOR PARietAL CORTEX MAY REPRESENT BASIS FUNCTIONS FOR SPATIAL TRANSFORMATIONS

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Many neurons in the monkey posterior parietal cortex have gaussian visual receptive fields that are modulated by a monotonic function of eye position, called the eye field. These receptive fields are more complicated in other parietal areas, in that they may vary with the direction of motion. In this field, single gain fields have been observed in neural networks that compute the head-centered position of objects (Gasper and Andersen, Nature, 1988), suggesting that populations of parietal neurons may form a distributed representation of the egocentric position of objects. There is an alternative interpretation for these gain fields in the context of sensory-motor transformations. Motor commands generated from the retinotopically organized eye position are nonlinear functions of these inputs. Such transformations can be approximated by using a simple intermediate representation in which neurons compute basic functions of eye position and the current eye position is known to form this intermediate representation. A gain field can be modeled as a gaussian function of retinal location multiplied by a sigmoidal function of eye position as a template for the pattern of the stimuli. In this way, the possibility that the posterior parietal cortex does not compute the positions of objects in a particular frame of reference but instead computes a general purpose representation of the retinal and eye position from which any transformation can be synthesized by direct projection. This representation predicts that hemineglect, a neurological syndrome produced by parietal lesions, should not be confused to egocentric coordinates but should be observed in multiple frames of reference in single patients, a prediction supported by several experiments.

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525.10

EXPLICIT SPATIAL REPRESENTATION IN PARIETAL CORTEX IS NECESSARY FOR PROPER CONJOINING OF VISUAL FEATURES

R. S. Friedman1,2,3, L. C. Robinson1,2,3, and A. T. Treue1,2,3.

1Center for Neuroscience, UC-Davis, CA 95616; 2Veterans Administration, Martinez, CA; 3Princeton University, Princeton, NJ.

Research with human and non-human primates implicates parietal cortex in spatial attention. Our studies of a patient with remarkably symmetrical, bilateral parietal lesions due to embolic infarcts suggest that spatial deficits may also contribute to impaired object perception.

The patient, RM, is frequently unable to make explicit spatial judgments. When asked to judge the position of a single object (left, right, or center, in one condition; up, down, or center in another), RM was incorrect for 95% of trials. This deficit in judging the location of one item was not further increased by the presence of an irrelevant distractor. However, if asked to judge the location of the first item relative to the second item, RM's performance fell completely to chance. Concomitant with these spatial deficits, RM has a tendency to miscompare features of objects. Errors of this type are referred to as "illogical conjunctions." For example, when presented with red and blue, he may report seeing a red O, even with free viewing (display times up to 5 sec) and undivided attention. Neurologically normal subjects produce illogical conjunction errors only under divided attention conditions with very short exposures. RM makes fewer illogical conjunction errors with sequential presentation of stimuli than with simultaneous presentation. In the absence of reliable spatial information, temporal information can be used to organize which features should be combined.

The data suggest that there are important interactions between the "what" and the "where" pathways. Spatial information plays an integral role in the construction of our perception of objects from sensory distributed information.
525.11

A MODEL FOR COMBINED EYE-HEAD TRACKING INCORPORATING THE PROPERTIES OF MSTI VT-NEURONS, P. W. Dietz and P. Thiel. Dept. of Neurology, University of Tübingen, 70570 Tübingen, Germany

Combined smooth eye-head-tracking (CEHT) is carried out by primate in order to stabilize the retinal image of a moving object close to the fovea. We have tested the idea that the generation of CEHT is based on a signal related to target velocity in space, reconstructed in a spatially distributed manner at the level of parietal area MSTI. Two 3-layered connectionist networks were used in order to represent area MSTI on the left and on the right side of the brain respectively. The input layer of each of the two "MSTI" networks was made up of units, sensitive to retinal image slip, eye velocity, eye velocity and head velocity with identical nonlinearities. Further experimental findings built into the networks were the speed-tuning of VT-neurons and the distribution of their preferred directions, characterized by the fact that every direction between 0 and 360 deg may be found on either side of the brain with a possible bias for ipsiversive directions. Using error backpropagation the "MSTI" networks could be trained successfully to output the sum and difference of the two networks equal target velocity in space. Assuming a fully activated VOR and furthermore taking into account the established properties of the eye and head motor systems, this simulates coordinated eye and head movements. The overall model has proven able to fit measured combined smooth eye and head movements under a wide variety of conditions. Furthermore it is able to predict the effects of experimental lesions of MSTI in monkeys: Unilateral MSTI lesions were mimicked by removing one of the two connectionist networks involved. This manipulation resulted in an ipsiversive eye-head tracking deficit, which, with respect to the eye movement part, is in full accordance with reported experimental findings. Supported by Graduiertenkolleg Neurobiologie Tübingen and DFG KFO "Neuroophthalmologie".

526.1


We are investigating the role of PLPs in the control of locomotion in Ascaris. API and AP2 were isolated from extracts of 10,000 heads. Confirmation of additional sequences has been limited by the low yield of purified peptide (2-10 pmoles).

We are now isolating PLPs from 1M male methanol extract of 5,000 heads and male tails. After 7 HPLC steps, 8 PLPs have been isolated and their sequences confirmed by MALDI-MS. The yield of purified peptide was 70-500 pmoles. Peptides API/AP2 range from 4-14 amino acids and have six C-terminal tetrapeptides: TFRF-, VLRF-, VLRF-, and PLRFamide. On the basis of their effects on muscle tension, 3 classes of bioactivity have been found.

This is the most heterogeneous family of PLPs reported from one species. Additional active peaks from this purification are being isolated at this time.

526.2

NEUROPEPTIDE PRODUCTS OF THE FMRFamide GENE MODULATE THE DROSOPHILA NEUROMUSCULAR JUNCTION. R.S. Hewes and P.H. Tzagkhi. Dept. of Anatomy and Neurobiology, Washington University Medical School, St. Louis, MO 63110.

The Drosophila FMRFamide gene encodes a complex, neuromuscular precursor protein encoding many diverse FMRFamide-related peptides. In the larval CNS, this gene is expressed in a small, stereotyped pattern of neurons, including 6 that project to the thoracic dorsal neural organs. From these FMRFamide-related peptides may be released to act on peripheral targets via the circulation. Three synthetic peptides—sequences deduced from the FMRFamide precursor (DPQDQFMRFamide, MDSNFMRFamide, SVQDNFMRFamide)—enhance nerve-stimulated contractions of internal and external abdominal muscle fibers by increasing peak tension, contraction rate, and relaxation rate. These peptide effects began within 1-2 min of the onset of peptide application, reached peak strength after 3-7 min, and ceased after 10-30 min. The threshold concentrations were 10 nM for DPQDQFMRFamide and MDSNFMRFamide and were 100-1000 nM for SVQDNFMRFamide. These results suggest that 1) receptors with high affinity for FMRFamide-related peptides are located on several skeletal muscles and/or on motorneuron terminals, and 2) multiple sequences contained in the FMRFamide precursor may be functionally equivalent at the neuromuscular junction. The design of labeled peptides will be important for receptor localization and for cloning of FMRFamide receptors by functional expression. Towards this end, we found that biotinylated peptide—enhanced nerve-stimulated contractions at a threshold of about 10 nM. (Supported by NIH Grants NS021749 and NS00701.)

We have used NADPH diaphorase histochrometry to establish the distribution of NADPH-oxidase in the feeding neurosecretory neurons of Lymnaea stagnalis. A dense population of stained fibers are present in the lip nerves and these are derived from a band of sub-epithelial bipolar cells located at the margins of the sensory neurons and NADPH may be involved in activating feeding. To test this hypothesis we used a semi-intact lip-CNS preparation where a sucrose stimulus can be applied to the lips while recording from identified neurons that control feeding. Sucrose activation of the feeding network was inhibited when the CNS was preincubated with the NO scavenger haemoglobin (Hb), the NO synthase inhibitor Nω-Nitro-L-arginine or the guanylyl cyclase inhibitor methylene blue (MB). Central activation of the feeding network by current injection into the slow oscillator (SO) interneuron was not affected by Hb. Application of NO donors or 8-bromo-cGMP to the CNS also activated the feeding network. We tested the behavioural significance of these physiological experiments by injecting animals with Hb or MB before placing them in a sucrose-rich environment and monitoring feeding behaviour. Both Hb and MB delayed the onset of feeding compared to met-Hb (which should not bind NO) and distilled water, respectively. Our results indicate that NO is released in the Lymnaea CNS by sucrose-activated chemosensory neurons and causes cGMP-mediated activation of feeding behaviour. This is the first description of a specific behavioural role for NO in the CNS. [Supported by BBSRC grant no. GR/J33234]


Prior to the development of cilia, Halisoma embryos exhibit a cilia-driven rotational behavior within the egg capsule. One component of this behavior, rotational surges, results from the transient stimulation of pedal ciliated cells by serotonin that is released from the identified embryonic neurons, ENC1. In order to examine the signal transduction mechanism underlying the serotonin-induced stimulation, ciliated cells were isolated from Halisoma embryos, plated under cell culture conditions and monitored in slow motion using time-lapse videomicroscopy. In the absence of serotonin, cilia beat spontaneously and regularly at frequencies ranging from 5-9 cycles per second. Serotonin induced a sustained, dose-dependent increase in cilia beat frequency with an EC50 around 1 μM. This stimulation was not mimicked by either potassium-induced depolarization or addition of 100 μM forskolin and 200 μM isobutylmethylxanthine, a treatment that elevates the level of intracellular cyclic AMP. In contrast, addition of thapsigargin (1 μM) or A23187 (10 μM), treatments that increase the concentration of cytoplasmic free calcium, caused an increase in cilia beat frequency similar to that produced by 100 μM serotonin. Furthermore, the response to serotonin was prevented when cells were exposed to calcium-free medium or the voltage sensitive calcium channel blockers, nifedipine or verapamil (1 μM). These data suggest that serotonin stimulates cilia beating by causing a calcium influx in a depolarization-independent manner.

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NEUROTRANSMITTERS

526.5


NADPH-diaphorase staining of the CNS of Aplysia was used to identify neurons that may contain nitric oxide synthase (NOS) and produce nitric oxide (NO). There are a few densely stained neurons in all ganglia and ca 30 bilaterally paired neurons in the ganglia 2 and 3 which can receive E-cluster terminals. Their axons and neurites are stained and form well defined fiber tracts and synaptic glomeruli, including the lateral terminus, which is also stained by antisem to myomodulin, a neurotransmitter / modulator. NADPH-diaphorase stains terminal processes on cell bodies of non-unstained E-cluster neurons and also in synaptic-like junctions. NO generated using 1 mM SIN-1 (3-morpholino-sydnonimine) and 5,7 (5-nitro-2-oxo-1,2-dihydropteridine) depletes these neurons, induces action potentials and increases input resistance. Degasued SIN-1 and SIN-C have no effects. A membrane permeant cGMP analogue, 8-bromo-cGMP mimics the effect of SIN-1 and SIN-C. E-cluster neurons stained by NADPH-diaphorase, and marked by dye injection, synaptically depolarize neurons that receive NADPH-diaphorase stained terminals. Consistent with NO effects, the response is calcium dependent and blocked by nitro-arginine. These results suggest that NO released from NADPH-diaphorase stained neurons acts like a neurotransmitter and depolarizes postsynaptic targets. cGMP may increase calcium cycles and increase cGMP, which alters ionic conductance.

526.7

NEUROTRANSMITTER 5-HT AND DA LEVELS AND THEIR SYNTHESIS ACROSS THE LIFE SPAN OF APLYSIA. C. Hong*, J. M. Flinn, R. Holt, V. Chandoke and Tom Capp.* George Mason University, Fairfax, VA 22030, *Univ. of Miami, FL 33149.

The levels of serotonin (5-HT) and dopamine (DA) in ganglion tissue were examined for animals ranging in age from 3 to 13 months post-hatch, the tissue tryptophan and tyrosine levels were also measured using HPLC. The results indicated that 5-HT increased slowly in the ganglion from 3 to 4 months and from 7 to 13 months, however it increased rapidly in the 4 to 6 month age range. In contrast, the increase in DA was predominantly constant across the age span. The ratio of 5-HT to DA showed a strong age dependency, with the lowest values at 3, 4, 12, and 13 months and the highest ratio at 6 months. 5-HT/DA represents the excitatory to inhibitory neurotransmitter ratio. These results suggest that DA may dominate 5-HT in very young and very old animals where previous studies have shown behavioral sensitization of the Gill-siphon withdrawal reflex to be weaker. The analysis of the ratios of neurotransmitter to its precursor in the ganglion (5-HT/TRP and DA/TRY) suggests that the synthesis of neurotransmitters may be one of the factors contributing to the changes in the net neurotransmitter levels. The 5-HT/TRP ratio increased at the 6 to 8 months period and then leveled off, while the synthesis of DA/TRY ratio had a slight increase after 7 months. Further studies on the metabolic enzymes and degradation courses are underway.

526.9

AGE RELATED CHANGES IN SEROTONIN AND DOPAMINE NEUROTRANSMITTERS AND RECEPTORS IN APLYSIA CALIFORNICA. M.D. Southal, S. Shirazi, V. Chandoke*, P. W. Royt and J. M. Flinn. George Mason University, Fairfax, VA 22030.

The age associated changes in dopaminergic and serotonergic receptors were examined in Aplysia californica. In earlier studies (Hong, 1994) it has been shown that the levels of serotonin (5-HT), dopamine (DA) and their ratio vary with age. In this study the levels of neurotransmitter receptors were examined in the ganglia obtained from 4, 5, 6, 10 and 12 month old animals. Receptor analysis was performed using radiolabeled bound ligands specific for the receptor of interest. [3H]-LSD was used to determine the saturation binding for total 5-HT and DA receptors. We report here the results for the 10 month (young) and 10 month (old) animals. Specific binding in the 5 month animals was found to be 9.9 fmol/mg of total protein for 5-HT and 8.4 fmol/mg protein for DA. For the 10 month animals, the specific binding for 5-HT was 20.9 fmol/mg protein and 16.4 fmol/mg protein for DA. This represents a two fold increase in binding for both 5-HT and DA in the 10 month as compared to the 5 month animals.

526.6


Vertebrate insulin can stimulate tyrosine autophosphorylation of a vertebrate insulin-like receptor in Aplysia bag cell neurons. We have therefore searched for peptides in Aplysia similar to vertebrate insulin using HPLC purification and a placental insulin-receptor autophosphorylation assay. Fractions from Aplysia embryos and total nervous system were purified by HPLC. One fraction both stimulates placental insulin-receptor autophosphorylation activity with insulin receptor-binding assays and in radioligand assays at nanomolar concentrations of the vertebrate peptide. The material in this fraction has been purified and its amino acid sequence is being determined. Initial results in the cerebral ganglion (Pergolizon et al., J. Comp. Physiol. A 164: 849), which contains cells that we may find to be homologous to the light green cells (LCGs) of Limynea (LGCS) produce mammalian insulin-like peptides – MIPs – which control growth and reproduction): We find that an antiserum against R15x2 peptide cross-reacts with vertebrate insulin as well as with the material we describe as insulin. Immunocytochemical studies with this antiserum stain two bilateral clusters of neurons in the cerebral ganglion that appear homologous to the LOC clusters in Limynea. Since these cells receive direct sensory afferents, this may be recognizing another peptide. As in Limynea, the neurons in Aplysia that exhibit this insulin-like immunoreactivity send processes to neurohaemal organs at the base of the lip nerves. Further studies are in progress to determine whether these LOC-like clusters are the source of the insulin that acts on bag cell neurons.

Neurons exhibiting cyclic AMP-gated current (I_{cAMP}) allow in vivo assay of cyclic AMP levels, synthesis and degradation (Huang and Gillette, J. Physiol. 98:831; 99:203; Sudhof, et al., in press). We have estimated 1) the concentration of CAMP due to stimulation by 5-HT, 2) 5-HT stimulated adenylyl cyclase activity, and 3) phosphodiesterase rate constants. Occillation of I_{cAMP} by 5-HT-induced current, and the voltage-dependent inactivation of I_{cAMP} and its coexpression with both CAMP and PKA and neuromodulators, indicate common modes of action. The procedure requires establishing: 1) the dose response relationship for I_{cAMP} with steady-state CAMP isophoretic current; 2) occlusion assay of I_{cAMP} with neuromodulator; 3) analysis of phosphodiesterase rate constants; and 4) measurement of cell diameter. Calculations yield an estimate of adenylyl cyclase activity per sec per unit membrane area. We have estimated the adenylyl cyclase activity in pedal ganglion G neurons in Pleurobrachia during 10 μM 5-HT stimulation to range from 0.98 to 2.77 pmol sec^{-1} cm^{-2}, averaging 1.59 ± 0.31 (n=5). 5-HT-induced CAMP concentrations at the membrane ranged from 4.94 to 12.14 μM, averaging 7.68 ± 1.22 μM (n=5). Supported by NIH grant ROI NS26588-01 to R.G.

AXON GUIDANCE II

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S27.1

OUTGROWING RETINAL AXONS GUIDED BY RADIAL (GLIA) CELLS. B. Schlosshauer* and R. Steinhauser. Max-Planck-Institut für Hirnforschung, West Germany.

In order to analyse the cellular mechanisms that govern oriented axonal outgrowth within the retina, we employed cytocryostats of the embryonic chicken. Visible retina strips were explanted onto cryosections of retina and axonal outgrowth was monitored. In vivo only ganglion cell axons and neurophilial axons (endfoot from the optic fiber) and radial Müller glia in the only nonneuronal cell type in the chicken retina. 1) Ganglion cell axons are directed by a dual mechanism: the retina tissue is composed of an inhibitory zone (cell layer) and an excitatory zone (optic fiber). In vitro, axon growth preferentially on the most external layer of retina cryosections even after coating sections with laminin. 2) Axons approaching outer retina layers respond partially with a growth cone (tempo time video recordings). In contrast, prior heat inactivation of cryosections allows axonal outgrowth even in outer retina layers. 2) The permissive zone is formed by endfoot of neurophilial/retinal glia. a) After elimination of ganglion cells and their axons due to prior optic nerve transection, explant axons still prefer the innermost retina layer in vivo pretreated cryosections. b) Isolated glia endfoot provide an excellent growth substrate. 3) The inhibitory zone is formed by somata of radial glia. a) Axonal outgrowth is inhibited on radial glia somata purified from retina cells cultured by complement mediated neuronal cytology. b) The restricted number of outgrowing axons on glia somata are highly fasciated therefore avoiding contact with glia somata (scanning electron microscopy). c) Heat inactivation of purified glia somata converts glia cells into a permissive substrate. The data suggest that ganglion cell axons guided by the innermost retina layer - polarized neurophilial/retinal glia cells with their endfoot being permissive and their somata being inhibitory for outgrowing axons. Supported by DFG.

S27.3

NEURONAL ORIGINATION OF THE EMBRYONIC FORE- AND MIDBRAIN OF MICE AT 8.5 DAYS' GESTATION. G.L. Mastick and S.S. Easter, Jr., Dept. of Biology, U. of Michigan, Ann Arbor, MI 48109.

The fluorescent axon tracers Dil and DiO were used on fixed E10.5 embryos to map groups of early neurones and pioneer axons with respect to interneuronal boundaries. The spatial and temporal relationship between central neurone and cranial nerve axonogenesis and spinal cord boundaries suggested a role of regional embryonic boundary. The fluorescing axon tracers injected at E9.5 and E10.5 were used to map the rostrocaudal, dorsoventral, and mediolateral coordinates of the rostral embryonic brain, revealing several general features: 1) Groups of neurones can be defined by major transverse interneuronal boundaries. 2) dmvXV and n8 somata were found in both mediolateral and rostrodorsal boundaries of the maxillary process, implying that these neuronal types are not simply defined by neurone migration or axon extension. In this axon transplant experiments, the somata of transplanted neurones were interposed with the very early dmvXV neurones in dorsal mesencephalon, and major axonal systems develop, marking the dorsal and ventral borders of the hindbrain. dmvXV (dorsal) and n8 (ventral) neurones were also projecting caudally into the rhombencephalon. Axons of the tract of the rostral commissure (TRC) extend from the base of the optic tract into dmvXV, suggesting that the TRC is dorsal, and the *front* end of the brain is ventral to the optic tract. 3) The pioneer axons do not follow interneuronal borders, instead, broad zones of the neural tube are filled with axons sharing trajectories. We examined the altered organization of the embryonic brain in Waclaw mutant mice using neuronal landmarks at E8.5. The loss of this secreted growth factor causes deletion of mesencephalon and rhombencephalon, 1, loss of ast and DiO, and creates a new boundary between the forebrain and rhombencephalon. 2. Surprisingly, pioneer axons navigate accurately across the new boundary.

S27.4

ARREST OF AXONAL GROWTH IN VITRO BY LIVING, SINCTIONED OR FIXED TARGET NEURONS. D.H. Barad* and B. Knud, Department of Anatomy and Neurobiology, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19129.

The regulation of axonal growth by CNS targets was examined using an in vitro system in which the extension of cerebelar mossy fibers from explants of basilar pontine nuclei is interrupted by their targets, granule neurons. This "stop-growing signal" is both target- and axon-specific, and its regulation involves the expression of NMDA antagonists, mossy fibers are not arrested by granule neurons, and the growth of (>300 μm) neurites is 3.4 times that of controls. NMDA has an opposing effect, causing the stop-growing signal, resulting in shorter mossy fibers. Neither NMDA nor its antagonists alter the growth of mossy fibers in the absence of granule neurons.

To determine if functional, pre- or postsynaptic NMDA receptors are involved in the stop-growing signal, mossy fibers were cultured on granule neuron monolayers and were perfused with various concentrations of NMDA agonists, paraformaldehyde, and on unfixed, frozen sections of cerebellum. In both cases, mossy fibers extended less than 100 μm from their explant, and the growth of granule neurons was also arrested. These results suggest that cerebelar mossy fibers compete for recognition sites on granule neurons. In addition, the results suggest that functional granule neuron NMDA receptors are not strictly required for the arrest of axons by target neuron, and if they are involved at all, they must regulate this process indirectly.
EVIDENCE FOR GUIDEPOST CELLS IN AXON OUTGROWTH DURING DEVELOPMENT OF LEEF. [Lee et al. 1992]. Department of Biology, Yale University, New Haven, CT 06511

The characteristic pattern of the bodywall innervation in Drosophila embryos provides an ideal system to study axon guidance and synapse formation. Segmentally reiterated (SNAs) grow out laterally from the anterior half of the segment and make a stereotypical posterior turn at mid-bodywall to innervate muscle 5 and 6. Cash et al. (J. Neurosci. 12:2501, 1992) showed that in the absence of these target muscle fibers, SNAs still turn posteriorly. Therefore, cues other than the targets are present to guide SNAs growth. Although SNAs trajectory can be altered by manipulation of the muscle fibers in its path, it always makes the posterior turn to innervate its target muscle fibers (Wang and Keshishian, Neurosci. Abstr. 18:1273, 1992). We observed the presence of 2 cells (possibly glia) at the corner of muscle fibers 12 and 5. Their locations correspond to the site at which SNAs usually makes ends on muscle fiber 5. In mutants of the spitz family, such as pointed, which affect ventrolateral patterning, 20% of the SNAs nerves fail to turn (Wang et al., Neurosci. Abstr. 19:235, 1993). Examination of pointed mutants showed: (1) In 6 out of 8 cases SNAs failed to turn when the 2 glial cells were missing. (2) In 20 out of 22 cases, SNAs turned normally when these cells were present. To test whether these cells are in fact guidepost cells, we are surgically removing them in filleted embryos at late stage 15, when SNAs has not yet turned. The embryos are then cultured until late stage 16, when SNAs normally has reached its targets. These experiments will critically define the role of these putative guidepost cells in inducing the posterior turn of SNAs.

SPECIFICITY OF SYMPATHETIC PREGANGLIONIC PROJECTIONS IN THE CHICK IS INFLUENCED BY THE MATURATION STATE OF THE SOMATIC MESODERM. L.W. Ying. Dept. of Neurobiology, University of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261

The spatio-temporal patterns of sympathetic preganglionic projections in the chick are segmentally specific. For example, T1 preganglionic neurons enter the sympathetic trunk at stage 27 and project rostrally. In contrast, T4 preganglionic axons enter the sympathetic trunk at stage 28 and project caudally. Previous studies from this laboratory have shown that the somitic mesoderm influences these patterns. This study examines whether the maturation state of the somitic mesoderm is responsible for this influence.

Two series of experiments were designed to confront preganglionic neurons with somitic mesoderm from older maturation stages 4 and 4 (homozygous embryo). The T4 neural tube segments of a stage 14 embryo were transplanted to the upper cervical (more mature) region of the same embryo. Orthodrome HER labeling showed that a subset of T1 preganglionic neurons translocated to the cephalic region occurred much earlier than normal, such that by stage 26 the translocated T1 preganglionic neurons have already contributed extensively to the sympathetic trunk. This indicates that preganglionic neurons will send their axons out earlier when they encounter a more mature somitic mesoderm. In another series of experiments, the T4 neural tube segments of a stage 11 donor were transplanted homotopically to a stage 18 host. In the resultant embryo, the T1 preganglionic neurons projected in both rostral and caudal directions, instead of in their normal, predominantly rostral direction. This result indicates that the direction of preganglionic projections can be altered by the maturation state of the somitic mesoderm.

The maturation of the somatic mesoderm may therefore relate to the formation of the specific pattern of preganglionic projections.

TESTING THE ROLE OF TARGETS IN MOTONEURONAL SPECIFICITY C.E. Beattie* and L.S. Efros, Institute of Neuroscience, University of Oregon, Eugene, OR 97403

We are investigating mechanisms underlying neuromuscular specificity in vertebrates using embryonic zebrafish. During zebrafish development, identification of motoneurons with specific somatic positions in the spinal cord becomes committed to innervate particular regions of myotome. Motor growth cones may be guided by motonal cues. Alternatively, they may grow in a specific direction regardless of motonal cues, suggesting that guidance cues originate from some other source. To differentiate between these mechanisms, we analyzed motor axonal outgrowth after altering the orientation of the myotome. Labeled donor myotomes were isolated, rotated along the dorsoventral axis and transplanted into unlabeled host embryos from which native myotomes had been removed. Following doronal-ventral reorientation of the myotome and the orthochronic transplantation at 16h, an identified motoneuron, CaP, extended its growth cone ventrally as it does normally. This result suggests either that doronal and ventral myotomal regions have become respecified or that CaP extends this axon ventrally independently of motonal cues. To resolve this issue, heterochronic transplants were performed. Following doronal-ventral reorientation and transplantation of 19h myotomes into 16h hosts, CaP extended an axon which often grew laterally and appeared to avoid the ventral myotomal region which now contained dorsal muscle. This result supports the idea that younger myotomes respect their dorsal and ventral regions while older myotomes retain their original polarity after transplantation. Motor axons, therefore, appear to be guided by cues originating from myotome. Supported by ACS PF-3982 and NS23915.
MOTOR AXON SPROUTS ARE INDUCED AND GUIDED BY PROCESSING OF NEUROMUSCULAR SYNAPSES TO FORM SCAGGLED NERVE AXONS IN THE POST-CONTRIBUTIONS TO THE MECHANISMS OF NEUROMUSCULAR JUNCTION REMODELING. C. C., B., W. L., T., and B. L. Thompson* and J.-Y. Soo. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

In the present study, we examined the possibility these cells play a role in the sprouting of motor nerves in adult mice.

We first examined nerve sprouting induced by partial denervation (PD). Within 3 days of PD, use of an anti-neurofilament antibody showed that nerve sprouts were extended about 28% of the terminal remains in the muscle. Staining with a SC marker (anti-S-100) showed that all of these sprouts were associated with SC processes. Staining with the antibody 4E2, which marks SCs at denervation, demonstrated that these sprouts extended from junctions which had been contacted by processes of SCs at denervation.

In the nerve sprouts grown along the SC process to the adjacent denervated endplate. These sprouts were observed to be confined to the endplate region and were often precisely aligned with motor nerve terminal branches. This alignment was not observed in neonatal mice. Following denervation, Schwann cells rapidly extended processes well beyond the endplate region, often joining with processes of cells from adjacent endplates, consistent with observations made using S-100 immunohistochemistry (Reynolds & Woolf, 1992). Recently, Thompson et al. have shown that following denervation, regenerating axons are closely associated with Schwann cell processes and suggest that these cells may play a role in axon growth guidance to the endplate (Son & Thompson, 2004). Observations of terminal Schwann cells in vivo will allow the dynamics of the response of these cells to denervation and their role in axonic regeneration and maintenance to be studied.


As discussed above, the implications of these results are that, in the context of the axon-stem cell relationship, our data suggest that the role of the stem cell in the regeneration of the axon is to maintain the balance of the autonomic and the behavioral systems. This is consistent with the findings of previous studies that have shown that the role of the stem cell in the regeneration of the axon is to maintain the balance of the autonomic and the behavioral systems.
528.3


Bolus injections of cholecystokinin octapeptide (CCK-8) are more potent for reducing sucrose intake when injected through an aortic catheter than when given intravenously (Callegari et al., Am.J.Physiol. 263:R572-R577). We compared suppression of intake of 30% sucrose produced by continuous near-ciliary and intravenous infusions of 100 - 1600 ng CCK-8. Adult, male Sprague-Dawley rats received infusions throughout 20 min feeding tests. Significant reductions in intake were produced by 400, 800, and 1600 ng near-ciliary CCK-8. Only the two highest doses reduced intake when administered intravenously. Behavioral observations indicated [1] that CCK-8 had no effect on feeding during the first several minutes but accelerated the decline in incidence of feeding later in the test and [2] that near-ciliary CCK-8 had more pronounced effects on feeding behavior than did intravenous CCK-8. Thus during slow infusions, greater potency of near-ciliary CCK-8 was accompanied by temporal intake patterns indicative of enhancement of satiety. The data provide further evidence that cholecystokinin acts within the upper gastrointestinal tract to inhibit food intake.

528.5


Neonatal treatment with monosodium glutamate (MSG) induces neuronal degeneration in the arcuate nucleus (ARC). This nucleus is the main hypothalamic site of neuropeptide Y (NPY) synthesis. Hypothalamic NPY preferentially stimulates carbohydrate intake and is influenced by diet composition. In this experiment, we measured the effects of a MSG treatment on NPY levels and dietary preferences. Newborn Long-Evans rats were injected i.p. with either MSG (4 mg/g BW) or saline on the 3rd, 5th and 7th days of life. Rats were fed on a control (C) diet until adulthood. Then, they could choose between a high carbohydrate (HC) diet and a high fat (HF) diet. NPY concentrations were measured in several microdissected hypothalamic nuclei. They were significantly smaller in the MSG rats than in the control rats in the ARC (- 52 %; p<0.001) and part of the paraventricular nucleus (pPVN) (- 57 %; p<0.004). Energy intake in the food choice situation was greater in the control than in the MSG rats (81.3 ± 5.3 vs 62.2 ± 3.7 Kcal/day; p<0.01). This augmentation was due to a greater intake of HP diet (> 60 %; p<0.001) and a smaller intake of HC diet in the control rats (- 25 %; p<0.01). These results confirm some of our previous findings showing that rats with a carbohydrate preference are characterized by lower NPY levels in the pPVN. Supported by Fondation B. Deleers (Paris).

528.6


Recent evidence shows that in vivo and in vitro NPY release in the paraventricular nucleus (PVN) and NPY gene expression in the arcuate nucleus (ARC) increase in association with hyperphagia in long-term diabetic rats. In the present study we sought the timing of the onset of NPY upregulation and hyperphagia in streptozotocin (STZ)-treated diabetic rats. Adult male SD rats were injected with either STZ (65 mg/kg) or vehicle via tail vein on day 0. Rats were killed on days 2, 3 and 4. PreproNPY mRNA levels in the medial basal hypothalamus which includes the ARC, were determined by solution hybridization/RNAase protection assay using a cRNA probe. NPY levels in the microdissected hypothalamic sites were also estimated by RIA in STZ-treated rats. As expected, STZ treatment induced marked hyperglycemia and hypoinsulinemia along with loss in body weight after STZ treatment. Analysis of NPY gene expression, peptide levels and food intake showed a remarkable temporal sequence of events. PreproNPY mRNA levels were significantly increased (4.5 fold, p<0.001) within 48 hours of STZ treatment. Similarly, the 7 hypothalamic sites examined, NPY peptide levels were increased only in the PVN 2 days after STZ injection. In contrast, food intake was decreased for 3 days and thereafter, a significant hyperphagia was evident on day 4. Collectively, these results show that the enhanced NPY synthesis and release in the ARC-PVN axis precedes hyperphagia in diabetic rats. Since very low levels of insulin can inhibit NPY release from the PVN nerve terminals (Soc. Neurosci. Abst. 19-1821, 1993), we propose that the regulation of food intake by insulin is mediated by NPY PVN interconnections of a subpopulation of neurons in the ARC. Supported by UP DSR KD 717 (AB); NIH DK7273 (BPK); VA Merit Review (CAS).

528.7

NPY IMMUNONEUTRALIZATION SUPPRESSES FEEDING IN VMH-LESIONED HYPERPHAGIC RATS. Sara P. Kral*, M.G. Dubey* and Pushpa S. Kalra. Departments of Neuroscience and Physiolog, Univ. of Fla, College of Medicine, Gainesville, FL 32610

It has been known for a long time that destruction of the ventromedial hypothalamus (VMH) induces hyperphagia and increase in body weight. The neural signal(s) responsible for the hyperphagic response have not been identified. Neuropeptide Y (NPY) is the most potent orexigenic signal known and the evidence that passive immunization of rats against NPY suppresses feeding is ample. We therefore tested whether NPY antibodies may act as a pharmacological appetite suppressor. Therefore, we hypothesized that hyperphagia in VMH-lesioned rats may be NPY-dependent. Female rats received electrolytic or sham lesions in the VMH and permanent cannulae in the third ventricle of the brain. The VMH-lesioned rats displayed robust hyperphagia (5.5 ± 1.1 g/2h) as compared to sham-lesioned rats (1.6 ± 0.3 g/2h) 12-26 days post-surgery. These rats were passively immunized against NPY by intraventricular injections of purified IgG containing a specific NPY-antibody (NPY-Ab) at 0900, 1100 and 1300 h, and food consumption was evaluated between 1300-1500 h. The results showed that food intake was suppressed by greater than 93% in the NPY-Ab injected, VMH-lesioned rats. Similar central injection of purified IgG from normal rabbit serum (control) had no effect on food intake in these VMH-lesioned or sham-lesioned rats. These results suggest that hyperphagia in VMH-lesioned rats may be mediated by NPY. The effects of NPY on feeding behavior are consistent with the view that NPY is an orexigenic signal. Preliminary results indicate that a specific NPY-antibody may act as a pharmacological appetite suppressor.
TAMOXIFEN PREVENTS NEUROLEPTIC-INDUCED WEIGHT GAIN AND HYPERPHAGIA IN FEMALE RATS. T. Baptista, E. de Baptista, M. Altmann, and R.S. Scott. Biological Psychiatry Branch and Clinical Neuroendocrinology Branch, NIMH, Bethesda, Md. 20892

Elsewhere, we have suggested that the weight gain and hyperphagia induced by chronic administration of neuroleptics in female rats is related to a neuroleptic-induced hyperprolactinemia, which causes a decrease in gonadal estrogen synthesis, ultimately resulting in decreased activity of hypothalamic neurons involved in satiety (Parada et al., 1989). This mechanism has been investigated using the D2 selective dopamine antagonist sulpiride, and the weight gain was prevented by concurrent administration of either estrogen or bromocriptine. Recent evidence suggests that the antiestrosten tamoxifen may act as an estrogen agonist on food intake regulation because, in the short term, it prevents weight gain and hyperphagia in ovariectomized rats. In addition, tamoxifen and neuroleptics compete for the same receptor in hypothalamic neurons (Gray et al., 1993), which further suggests that tamoxifen may prevent neuroleptic-induced weight gain. To assess this hypothesis, female rats were divided into groups of 10 animals each, and were treated for 21 days with either vehicle, sulpiride (20 mg/kg, i.p.), or sulpiride plus one of the following doses of tamoxifen citrate: 2.5, 5, 10, 20, 50, or 100 mg/kg, i.p. Sulpiride-induced weight gain and hyperphagia were completely prevented by tamoxifen at doses of 5 or more mg/kg. A trend was observed between the dose of tamoxifen and inhibition of weight gain under sulpiride: r = 0.68, F(1,6) = 4.4, p < 0.08. Tamoxifen may be useful to prevent weight gain in obesity prone women under neuroleptic treatment.


Feeding can increase extracellular dopamine (DA) levels in the nucleus accumbens (NAc) of freely moving rats (Hernandez & Hoebel, Physiol. & Beh. 44, 599), but little is known about the effects of obesity on basal DA release in the NAc. In the present study, female albino rats (n=16) developed cafeteria-diet obesity and body weight averaging 23% above controls (n=16). In vivo microdialysis showed that basal DA levels in the NAc of the obese rats were 70% lower than in controls (p<0.01), but d-amphetamine (1.5 mg/kg i.p.) increased NAc DA to 75% of basal levels in the obese group and only to 25% in the control group (p<0.05). When both control and obese rats were food deprived for 24-36 hrs, there was no longer any measurable depression of basal NAc DA in the obese group. After a Purina pellet meal, NAc DA increased significantly in the food deprived controls (p<0.01), but not in the food deprived obese, however, a cafeteria-diet meal did increase DA (p<0.01). The results suggest that cafeteria-diet obesity (a) dampens basal activity of the mesoaccumbens DA system, but mild food deprivation and highly palatable food restore it, and (b) increases the amount of DA available for release by amphetamine.

Support by USPHS grant NS 30697.


Hyperphagia following fasting is a robust phenomenon. However, the actual signal used by a fasted animal to detect the necessity to ingest calories is still not known. Theories have focused upon glucose or lipid oxidation and cellular energy generation. We conducted the following study to investigate the relationship between several whole-body metabolic parameters and hyperphagia following food deprivation.

Recent long-term rats (14) were food deprived for periods ranging from 3 to 16 hours. Two hours before refeeding, they were placed in an indirect calorimeter for 2 hours. The rats were then returned to their home cages, food was restored, and food intake was recorded for 3 hours. Multiple regression analysis of several parameters, including respiratory quotient, carbohydrate and fat oxidation rates, energy expenditure, body weight, VO2, and VCO2 was performed. Of all the variables considered, only carbohydrate oxidation rate reliably predicted the amount of food consumed when access to food was restored. Importantly, using linear regression line fitting, a function was derived which predicted 0 grams of intake at a carbohydrate oxidation rate identical to that observed in the post-prandial state. These data indicate that fasting carbohydrate utilization rate may be an important determinant of refeding hyperphagia.

ANTISENSE OLIGODEOXYNUCLEOTIDES TO G-PROTEIN SUBUNIT SUBCLASSES: CHRONIC INTRACEREBROVENTRICULAR (ICV) INJECTION INDUCES A PHARMACOLOGICAL MODEL OF HUNGER. T. M. Hirsch-Multer, 1,2HLS, Univ. Delaware, Newark, DE 19716 and Dept. of Pharmacology, Zeneca Pharmaceuticals Group, Wilmington, DE 19897

Heteromeric guanine nucleotide-binding (G) proteins transduce signals from the membrane G-protein-coupled receptors to cellular effectors. We studied rats the effects of the chronic ICV (into the third ventricle) infusion for 72 h (through osmotic minipumps) with antisense phosphoetho-oligodeoxynucleotides (15 pg/24 h) corresponding to G-protein subunit subclasses (a, B, and G) The results for nighttime food intake (mean±SE, g) at the 48-60 h period after initiation of the vehicle, antisense or sense infusion were as follows: 20.2±1.1, 10.7±2.2, and 20.4±1.1, n=8; Ga3 antisense (to Ga11, 12 and 13), 20.0±1.1, n=8; Ga3O3 common antisense (to Ga10 and Ga2B), 15.8±1.0, p<0.01, n=11 (water intake increased 9% from control); Ga2B antisense, 15.4±1.1, p<0.01, n=11; Ga3O3 antisense, 19.2±1.1, n=10; Ga3 antisense, 15.4±1.1, n=7; Ga2B common antisense, 20.3±1.1, n=5; Cy3 common antisense, 15.8±0.5, p<0.02, n=6. (Cy3 1, 2, 3 and 4 antisense also decreased feeding, n=6-7/group). Infusion with 15 µg/24 h of Ga3O3 common sense or Ga2B sense had no effect on nighttime food intake (19.4±1.1, n=8, and 20.7±1.1, n=9). Computerized analyses of behavioral patterns demonstrated that the Ga3O3 antisense depresses nighttime feeding by reducing meal frequency (to 4.9±0.3 meals, n=11, p<0.001 from control: 7.5±0.6 meals, n=8) without decreasing meal size or meal duration. Immunoblots of hypothalamic ventromedial nucleus from normal rats (n=12) show that the levels of Ga3O3 protein subunit are >50-fold higher relative to those of Ga11 and Ga2B subunits. The results suggest that the G-protein subunit subclasses Ga3O3 and Ga3 are required for the modulation of normal feeding behavior, and that changes in their activity may be associated with the chemical regulation of feeding.

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529.1 CROSS-TOLERANCE TO D, BUT NOT D, Dopamine Receptor Agonist ON Rats TOLERANT TO CAFFEINE-INDUCED ROTATIONAL BEHAVIOR. R.E. Garrett/Pand S.G. Holtzman. Dept. of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322.

Caffeine produces contralateral turning in rats with unilateral 6-OHDA (8 μg/μl)-induced lesions of the nigrostriatal pathway. The present study a) determined if tolerance develops to this effect of caffeine and b) examined the effect of dopamine receptors. Male Sprague-Dawley rats with 6-OHDA lesions received scheduled access to either a 0.1 % caffeine solution (average drug intake = 70 mg/kg/day) or to drug-free tap water (controls). The rats were subsequently SC injected with 5 mg/kg caffeine and turning was recorded for 3 hr. Caffeine (100 mg/kg) and theophylline (3-100 mg/kg) produced biphasic increases in contralateral turning in control rats, with the peak effect being 0.3 to 10.5 mg/kg, respectively. Rats receiving caffeine chronically were tolerant to this effect of both drugs. The selective D1 agonist quinpirole (0.01-1.0 mg/kg) and R(-)-propylnorapomorphine (NPA, 0.003-1 mg/kg) and the selective D3 agonists SKF-38393 (0.3-10 mg/kg) and SKF-77434 (0.3-10 mg/kg) also produced dose-dependent increases in contralateral turning in control rats. This effect of the D3 agonists was reduced significantly in rats receiving caffeine chronically, whereas the effect of the D1 agonists was unchanged.

Therefore, tolerance develops to caffeine-induced rotational behavior and there is cross-tolerance to the methylphenidate derivative theophylline. The differential cross-tolerance among dopamine receptor-selective agonists supports the claim that tolerance to caffeine-induced rotational behavior is mediated by the D3, but not the D1, dopamine receptor. (Supported in part by DA03413, K05 DA00008 and P.R. Harris Pre-doctoral Fellowship).

529.2 Neurobiological bases of methamphetamine action in the hippocampus, E.S. Onaitis1, K.A. Parker2, A. Chapkarian1, G. Chapulhus1, E.D. Motley3, C. Bishop-Bobinson1 and S.S. Citrona. 1Depts of Pharmacology, 2Physiology and 3Division of Biomedical Sciences, Meharry Medical College, Nashville, TN 37208.

The effect of psychoactive drugs such as psychomotor agitation, attentional disturbances and memory dysfunctions suggests the involvement of the hippocampus and temporal lobe. However, the exact mechanisms by which the above effects are elicited are not known. A multidisciplinary approach is being used to investigate the neurochemical bases of behavioral changes induced by methamphetamine. This involves following function after administration by methamphetamine in the hippocampus of a rodent model. Groups of rats (N=8-12 per group) were administered with vehicle, 1.0 and 10.0 mg/kg methamphetamine (ip) for about 3 months. The locomotor activity, stereotype behavior and performance in the elevated plus-maze tests were evaluated weekly. A reduction in exploration to the open arms was produced by the 1 mg/kg dose. The animals treated with the 10 mg/kg dose displayed a reduced attention which reverted to an intense movement towards the open arms of the maze after six weeks of treatment. Locomotor activity and stereotype behavior were significantly increased following treatment with the low and high doses of methamphetamine. The magnitude being greater at the 10 mg/kg dose. The tolerance which developed was more pronounced with the locomotor activity and stereotype behavior than with the performance on the phono-gradient. The neurochemical, molecular and electrophysiological bases underlying these neurobehavioral changes induced by methamphetamine are currently being studied in the hippocampus and cortex by NSF-MCB- Grant R01 HD325157 and RCMI Grant NIH S10RR013208.

529.3 BEHAVIOUR AND NEUROCHEMICAL STUDIES OF A PHENETRINE AND FENFLURAMINE DRUG MIXTURE IN RATS. Robert Motley, E.T. Logan, H.K. Azmitia and R.P. Motley. Department of Neurobiology, University of Texas Southwestern Medical School, Dallas, TX 75235.

Clinical case studies suggest that combined administration of phentrenine (PHEN) and fenfluramine (FEN) may be useful in the treatment of alcohol and cocaine addictions (Hitzyg, Maryland Mech J 42, 1993). The present experiments examined the nature of interaction between the two amineogenic drugs using the drug discrimination paradigm to examine subjective effects and the in vivo binding studies technique to assess the neurochemical interactions in the nucleus accumbens. Three groups of Sprague-Dawley rats were trained to discriminate saline from (1) FEN (1.0 mg/kg i.p.), (2) PHEN (1.0 mg/kg i.p.) and (3) PHEN+FEN (1:1 ratio) under a fixed ratio (FR10) schedule of food reinforcement. The majority of rats trained on the mixture acquired the discrimination after 32±4 sessions. In comparison the majority of rats trained to discriminate the individual compounds failed to meet criteria in 60 sessions. The individual compounds (1 mg/kg i.p.) generalized partially to the mixture, and complete generalization was observed following 3.0 mg/kg of FEN or PHEN. In concuss rats, local infusions of FEN (1aM) or PHEN (1aM) into the nucleus accumbens selectively increased serotonin and dopamine respectively. The mixture (1:1 ratio) increased both amines to a similar degree. Therefore, the dual stimulation of the amines by the mixture may be the basis for the cueing effects of the FEN+PHEN drug mixture. Furthermore, these amineERICs appear to interact additively as well as behaviourally and neurochemical levels.


Cocaine and methenylidate (MP) have very similar pharmacological properties. Yet cocaine is considered one of the most reinforcing and addictive drugs while MP is widely used to treat attention deficit disorder in children. PET was used in conjunction with [11C]methenylidate to measure the pharmacokinetics and regional brain distribution of methamphetamine in normal volunteers. The relationship between MP's pharmacokinetics and MP, induced "high" were also evaluated. The results were compared with those previously obtained for cocaine as well as with those obtained with [11C]cocaic. MP's absolute uptake in brain (7±10%) as well as its regional distribution, was identical to that of cocaine. MP pretreatment (0.5 mg/kg i.v) inhibited binding in striatum suggesting, that as for cocaine, specific binding was limited to striatum. The pharmacokinetics of MP and cocaine in brain were significantly different. Clearance of MP from striatum (90 minutes) was significantly slower than that of cocaine (20 minutes). For both drugs their fast uptake in striatum paralleled the experience of the 'high'. For MP this 'high' decreased very rapidly despite significant binding of the drug in brain. In contrast for cocaine, the decline in the high paralleled its fast rate of clearance from brain. Because the experience of the 'high' correlated only with the initial fast uptake of the drug in brain, the slow clearance of MP from brain may serve as a limiting factor in promoting its frequent self-administration and may explain the much slower abuse of MP than of cocaine.


MDMA and fen are substituted amphetamines that bind to the serotonin transporter, promote the release and inhibit the re-uptake of serotonin in pre-synaptic terminals. The release of serotonin and inhibition of its uptake and catabolism may contribute synergistically to raise extracellular levels of serotonin in the brain. A consequence of increased extracellular serotonin is PKC activation (see abstract by Kramer and Azmitia) and the stimulation of glycogenolysis (see abstract by Poblete and Azmitia) which may contribute to the neurotoxic mechanism of substituted amphetamines. We now report that MDMA and FEN at toxic concentrations ranging from 1-10 μM potentiates the effect of clorgylline (10 μM) on the release of serotonin. The clorgylline potentiation of the MDMA (10 μM) toxicity is concentration dependent. Approximately 1 because the effect of the MDMA is a potentiation of the serotonin transporter in a dose-dependent manner. Serotoninergic cells were obtained from E4 rat pups. A rostral monothecalous and caudal hypothalamic and cortical regions were obtained and each was treated with clorgylline. Following rinsing and further drying, slices were plated on poly-D-lysine coated well plates (1x106 cells/mL) and were incubated at 37°C. Full was added on day 2, and was removed followed by the Proliferation of serum free media on day 3. Drugs were added on day 8 and uptake assay of 1H5-HT was performed on day 12. Samples were counted by liquid scintillation counting at a counting efficiency of 58%. These results suggest that serotonin may be a component in the neurotoxic mechanism of MDMA and FEN. This work was supported by NIDA contract #271-87-8144 to E.C.A.

529.6 THE ACTIVATION OF PKC BY MDMA IS DEPENDENT ON THE RELEASE OF 5-HT FROM VISIBLE NERVE TERMINALS. H.K. Kramer and E.C. Azmitia, Dept. of Biology, New York University, NY, NY 10003.

Substituted amphetamines, such as 3,4-methylenedioxymethamphetamine (MDMA), have been shown to bind with high affinity and to inhibit the serotonin (5-HT) uptake transporter, promote the release of 5-HT, increase the uptake of extracellular Ca2+, and inhibit the activity of monoamine oxidase. Additional studies have shown that MDMA's acute and neurotoxic effects arise from an interaction with the central 5-HT receptor. This receptor is coupled to the breakdown of membrane-bound phospholipids and the activation of protein kinase C (PKC). These effects, occurring concomitantly, appear to underlie MDMA's neurotoxic properties. Our laboratory has previously reported that MDMA induces a prolonged translocation of soluble PKC to the plasma membrane when administered in vivo, and this persists up to 5 days after the consecutive treatment. We now report that this metabolic effect is dependent on the release of 5-HT and the activation of postsynaptic serotonin receptors. Male Sprague-Dawley rats were exposed to MDMA (8 ± 20 mg/kg, s.c) and assayed for PKC translocation. A subset of animals were pretreated (21 days earlier) with the 5-HT neuromodulator, p-chloroamphetamine (PCA), to reduce cortical 5-HT content. 72 hrs following the last MDMA injection, rat cortices from both groups were assessed for PKC translocation and 5-HT terminal density via high affinity 5H2-PG and 5H2-pantetheine binding, respectively.

MDMA induced a lasting translocation of PKC in animals exposed for days 4 (saline 7.2 ± 0.25 pmol/mg wet prot: MDMA 10.7 ± 0.37 pmol/mg prot.; p<0.05). Rats pretreated with PCA showed a significant (49.54%) decrease in the density of cortical 5-HT uptake sites, and in those groups, MDMA was unable to induce PKC translocation. We also found that the prior loss of cortical 5-HT nerve terminals prevents the cytosolic release of 5-HT by MDMA and the concomitant activation of PKC. These results show that the presynaptic release of 5-HT mediates this metabolic effect of MDMA and that the prior loss of phosphorylation of its membrane-bound substrates may contribute to amphetamine-induced neuronal death. (NIDA #271-87-7043 to ECA)
S9.7

ACTIVATION OF GLYCOCEN PHOSPHORYLASE BY MDMA(+1) IN ASTROGLIAL-RICH PRIMARY CULTURES. J.C. Pilbes and E.C. Azmitia. Dept. of Biology, New York Univ., NY 10003.

Glycogen is the single largest energy reserve in the brain. Neurotransmitters, neuropeptides, and ions regulate glycogen levels in the brain by modulating the activity of glycogen synthase (GSase) and glycogen phosphorylase (GPase). GPase has recently been shown to be localized with glial fibrillary acidic protein (GFAP), an astroglial marker, suggesting that glycogen is localized in astroglial cells. Additionally, serotonin (5-HT) has been shown to stimulate glycogenolytic and functional receptors are found in neurons and in glia. It has been reported that 3,4-methylenedioxymethamphetamine (MDMA), a drug of abuse, stimulates the release of, inhibits the uptake of, and selectively inhibits the activity of MAO-A. This biochemical consequence of MDMA results in an increased 5-HT activity. This study investigates the effects of MDMA and 5-HT on glycogen metabolism in the CNS. A histological method was designed to visualize active GPase in an astroglial-rich primary culture. The number of GSase-reactive sites was measured using computer-aided densitometric software (OPTIMAS, Bioscan, Inc.).

5-HT activates GPase in a concentration-dependent manner. Maximal activation by 5-HT was achieved at 10 nM and resulted in a 118% increase in the number of reactive sites. Interestingly, MDMA+1 directly stimulated GPase activity with maximal activation induced by 5 nM and which caused a 70% increase in the number of reactive sites. The effects of 5-HT and MDMA+1 were abated by 50% in the presence of a 5-HT (S2) receptor antagonist. Our results indicate that these effects may be mediated by 5-HT receptor and that MDMA leads to increased synaptic 5-HT activity. These effects may be mediated by 5-HT receptors and that MDMA leads to increased synaptic activity. Thus, the increased energy state of the synapse will lead to a decrease in the glial-dependent membrane uptake contributed to substituted amphetamines. An astroglial-neuron metabolic link may be vital for synaptic homeostasis (NIDA 271-90420).

S9.9

Administration of methylenedioxamphetamine (MDA) to rodents causes loss of monoaminergic terminals. The mechanism by which MDA causes its neurotoxicity is not known. MDA might kill these terminals by producing oxyradicals due to the increased release of dopamine in the synaptic space (Nash and Nichols, 1991). In order to further evaluate the role of oxyradicals in MDA-induced neurotoxicity, we have tested its effects in transgenic mice (SOD-Tg) which overexpress the human SOD gene. In nontransgenic mice, MDA administration causes a significant loss of DA terminals in the caudate-putamen measured by receptor autoradiography of [125]IjP11-T3S-labeled dopamine uptake sites. In contrast, there were no significant changes in heterozygous SOD-Tg mice. Homozygous SOD-Tg mice show small decrements in striatal DA terminals which were of much smaller magnitude than those observed in the Non-Tg mice. These results suggest that MDA-induced toxicity in rodents may be secondary to the increased production of superoxide radicals after administration of MDA.

S9.11

In this study, the effects of a single dose of the indirect dopamine agonist amphetamine (AMPH) and methamphetamine (METH) on mRNA expression of c-fos, zP268 and preproyotrin (PpD) in various regions of rat forebrain were investigated with quantitative in situ hybridization histochemistry 1, 2, 3, 6 or 30 h after injection. A different and more intensive behavioral syndrome was induced following METH (5 mg/kg, i.p.) as compared with that observed after AMPH (50 mg/kg, i.p.). METH induced a far greater increase in c-fos and zP268 mRNA in sensorimotor cortex, dorsal striatum (caudoputamen) and ventral striatum (nucleus accumbens) than did AMPH. The increase in c-fos mRNA expression peaked at 1 h and returned to basal levels in all areas at 3 h. In contrast, the increase in zP268 mRNA expression in the cortical regions was equally strong at 1 and 2 h, gradually returning to basal levels by 6 h after drug administration. However, in the striatal regions, zP268 mRNA levels peaked at 1 h and declined gradually to basal levels by 6 h. Interestingly, METH caused an actual suppression of zP268 gene expression (50%) both caudate and nucleus accumbens at 3 h. PpD mRNA expression was increased in a patchy fashion in the caudoputamen and nucleus accumbens beginning 2 h and returning to basal levels by 30 h after injection of either drug. This study, together with our recently published observation that PpD mRNA is induced in the caudate 3, 6, and 18 h after AMPH or METH injection (Smith and McCarty, Mol. Brain Res., 21:359-365, 1994), provides a detailed description of the differential regulation of c-fos, zP268, and PpD mRNA expression in the cerebral cortex and striatum by amphetamines over time (Supported by DA03982).

S9.8

MDMA-INDUCED NEUROTOXICITY IN RAPHE PRIMARY CULTURE INVOLVES LARGININE-NO AND OXIDATIVE PATHWAYS. C. Cerulli, P. Sheng, B. Landeheim, and J-L. Cadet. Molecular Neurophychiatriy

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Methylenedioxymethamphetamine (MDMA) is a neurotoxic drug of abuse. Its use is also associated with long-term depletion of serotonin that correlates with morphological damage to serotonergic terminals. The mechanisms by which these glial described sites of action have yet to be elucidated. It has been pointed out that free radical-mediated events may form a final common pathway in neurotoxic processes. In order to test the role of the free radicals, nitric oxide (NO), and a model of serotonergic cells cultured from raphe nuclei of rat fetuses. In these cells, MDMA causes dose-dependent cytotoxicity. Cell death was attenuated by the NO synthase inhibitors, nitro-L-arginine and L-nitro-methylarginine. Moreover, the toxic effects of MDMA were attenuated by benzamidine, an inhibitor of ADP-ribosylation. Primary cultures of serotonergic cells obtained from SOD-transgenic mice were also protected against MDMA-induced toxicity. These results suggest that MDMA induces neurotoxicity via NO formation and by the production of superoxide radicals.
3.1
HLM MRI scan shows sparing of the posterior half of the hippocampus and parahippocampal gyrus S. Corkin, D. G. Amaral, K. A. Bourgeois, W. Crooks, and the Memory Disorders Research Center, MIT, Cambridge, MA, 02139; Center for Behavioral Neuroscience, SUNY, Stony Brook, NY, 11794; Dept. of Radiology, Brigham and Women's Hospital, Boston, MA, 02115; and Dept. of Neurology, Massachusetts General Hospital, Boston, MA, 02114.

3.2
Evidence of intact perceptual priming with novel stimuli (patterns, pseudowords) in amnesic subjects suggests that they may be able to learn novel information when tested with other repetitive tasks. To test this hypothesis, two groups were examined word-stem completion priming with novel words in the amnesic patient H.M. (age = 67 yrs, ed. = 12 yrs) and in normal aging and age-education-matched control subjects (NCS). Subjects were tested on word-stem completion in priming, control, and a vocabulary test (four-alternative forced choice). The stimuli were common English words that entered the dictionary after 1965, and thus came into general usage after the onset of H.M.'s amnesia, i.e., words for which he presumably learned the meaning. Axial and concave T1-weighted (TR=550 msec, TE=16 msec) and T2-weighted (TR=2500 msec, TE=90 msec) images were obtained with a 1-mm slice thickness and a 1-mm interval between slices (matrix=256 x 192 pixels; NEX=1; FOV=22 cm). The bilateral lesioning of the temporal pole, angular, and entorhinal cortex. Approximately the anterior 2 cm of the dentate gyrus, hippocampus, and subicular complex are examined, but the posterior 2-3 cm of these fields are intact but not visualized. The medial parietal cortex is also examined, but because the collateral sulci and its medial cortical are visualized, at least some of the posterior perisylvian and parahippocampal cortex is intact. Neocortical atrophy is slight and a matter of age.

3.3
COGNITIVE PROCESSES UNDERLYING RECOGNITION MEMORY: EVIDENCE FOR A SPECIFIC IMPAIRMENT OF RECOLLECTION IN AMNESIA A.D. Wagner, M. Verfaillie, P. Croce, and J.E. Gabrieli, Dept. of Psychology, Stanford University, Stanford, CA 94305; and the Memory Disorders Research Center, Boston University School of Medicine and DVARC, Boston, MA 02130.

Studies of recognition performance in normal subjects suggest that two separable processes mediate recognition memory: controlled recognition and automatic fluency. We have hypothesized that recognition failures in amnesia (AMN) reflects a disproportionate impairment of recognition and that fluency may be spared. We examined the status of recollection and fluency in AMN by equating the recognition memory performance of 8 AMN patients and 11 control (CON) subjects. CON subjects were presented single study and yes/no recognition test trials for separate lists of high-frequency (HF), medium-frequency (MF) and low-frequency (LF) words. For each list, AMN patients had the same initial study and test trial followed by seven additional study-test sequences with the same words. Neutral distractors in each test trial. AMN subjects had better recognition than AMN patients in the first trial and also showed superior recognition accuracy for LF versus HF words. The additional study-test trials equated AMN patients' recollection accuracy with that of CON subjects for HF words, but failed to equate the same patients' recognition of LF words. A separate study with 96 college students indicated that recollection may play a greater role in recognition of LF than HF words. Taken together, these results suggest that the improved recognition performance exhibited by AMN patients following extra study-test trials reflects fluency processes and not recollection processes. These findings indicate that the processing basis of recognition performance in AMN patients shows extra study is not the same as that of CON subjects, even when recognition accuracy is numerically equaled, and that recollection, relative to fluency, is disproportionately impaired in AMN. Supported by grants from the NSF and NIH (NINDS 1P01NS026685 and NIA RO1AG1121).

3.4
The core memory deficit in amnesia is neither one of conceptual processing nor one of explicit retrieval. C. Vyas, J. B. Dem, M. M. Kinsar, L. A. Morse, and J. H. Eichenbaum, Dept. of Psychology, Stanford University, Stanford, CA 94305; and the Memory Disorders Research Center, Boston University School of Medicine and DVARC, Boston, MA 02130; Dept. of Neurological Sciences, Rush Medical College, Chicago, IL 60612; Beckman Institute, Univ. of Illinois, Urbana-Champaign, IL 61801.

Amnesic patients' memory is often intact on implicit tasks, despite severe deficits on explicit tasks. Because most implicit tasks depend on perceptual processing, and most explicit tasks on conceptual processing, it has been proposed that the core memory deficit in amnesia is one of conceptual processing. By this view, amnesic patients should be impaired on both implicit and explicit tasks that require conceptual processing, and intact on both implicit and explicit tasks that require perceptual processing. To test this view, 23 amnesic and 23 control subjects performed four tasks: implicit-perceptual (word fragment completion), implicit-conceptual (word fragment association, experimental word subsequence targeted, and control word subsequence targeted), and explicit (word concept association, word concept recall, and explicit-conceptual (word concept recall). A study-phase modality (auditory/visual) manipulation varied the nature of the processes (perceptual and conceptual) engaged by each task. Amnesic patients were impaired on both implicit and explicit tasks (perceptual and conceptual) and intact on both implicit tasks. Thus, the core memory deficit in amnesia is not one of conceptual processing. Alternatively, amnesia may reflect impaired declarative processes, rather than impaired explicit retrieval per se, because explicit tasks require processing of declarative or relational information. By this view, amnesic patients should be impaired on any task that requires declarative processing, regardless of the retrieval instructions. To test this view, 10 amnesic and 11 control subjects performed the general knowledge task, under implicit and explicit retrieval instructions. Amnesic patients were impaired under both implicit and explicit instructions. Results are consistent with multiple memory systems views postulating that amnesia reflects a selective impairment in a limbic-diencephalic memory system that is engaged during declarative processing.

3.5
DISSOCIATION BETWEEN FORMS OF ASSOCIATIVE IMPLICIT MEMORY THAT ARE INTACT OR IMPAIRED IN GLOBAL AMNESIA A. D. Loo, J. L. DeR, E. B. N. Reminger, and W. S. Franc, Dept. of Psychology, Stanford University, Stanford, CA 94305 and Northwestern University, Evanston, IL 60208.

Patients with amnesia have a deficit in learning new associations between words when memory is measured by explicit tests. Amnesic patients have a more variable pattern of results when learning new associations that each new association is measured by implicit tests. They are impaired when the implicit test is one of stem completion (SC), but they have shown normal learning when the test is one of reading speed (RS) or perceptual identification (PI). The present study examines whether these patients show a similar dissociation between different measures of implicit memory for new associations. In the first study, subjects either read aloud word pairs or generated sentences. In the test phase, subjects performed either a SC or a PI test with word pairs identical to those studied (same context), recombinations of studied words (different context), and new items (baseline). The measure of learning is the difference in learning between same-context and different-context pairs. For SC priming, associative learning occurred only if subjects performed the more demanding sentence-generation task. In contrast, subjects showed associative PI priming after both reading and sentence-generation study tasks. Additional studies examined associative priming by measuring by RS. In one experiment, subjects read aloud with and without instructions in the study phase. While explicit memory was superior in the imagery condition, associative priming was similar in both conditions. Further experiments indicated that associative priming, as measured by RS, does not depend on both a perceptual component (it was diminished when words were studied auditorily and tested visually) and a productive component (it was eliminated if subjects read the word pairs aloud in study phase). Results indicate that different implicit tests of the learning of new associations provide measures of dissociable mechanisms. The PI and RS tests may reveal perceptual and productive associative mechanisms that do not and that do not depend upon declarative memory processes. SC tests may reveal lexical or semantic associative mechanisms that require elaborative study and that depend upon declarative memory processes. Supported by grants from the NSF and NIH.

3.6

Functional mapping of implicit memory was examined via electrical stimulation of subcortical structures implanted in the superior temporal lobes of epileptic patients. All patients were undergoing speech mapping prior to temporal lobe resection for treatment of intractable seizures. Membrane electrodes were implanted as subjects performed language tasks such as confrontation naming of single pictures and identification of objects from an array of four pictures. Auditory presentation of memory materials occurred as patients performed an auditory responsive naming task (e.g., answering questions such as "Tell me what you carve at Halloween"). These auditory stimuli were either nonword echolalic words or word fragments targeted for implicit retrieval (2-15ms) was delivered periodically to selected brain sites as patients performed the tasks. Implicit memory was assessed as subjects performed a category membership evaluation. In this task, patients were asked to name three items from a given category and priming was observed when patients were more likely to provide target items recently presented during the language task. Implicit memory was assessed as subjects performed a category membership evaluation. In this task, patients were asked to name three items from a given category and priming was observed when patients were more likely to provide target items recently presented during the language task. Implicit memory was assessed as subjects performed a category membership evaluation. In this task, patients were asked to name three items from a given category and priming was observed when patients were more likely to provide target items recently presented during the language task.

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530.7 PET ACTIVATION MEASURES REVEAL A DISASSOCIATION BETWEEN BRAIN REGIONS UNDERLYING PERCEPTUAL AND CONCEPTUAL PROCESSES IN PICTURE-NAMING PRIMING. S. M. Park*, T. A. Blaxton, J. D. E. Gabrieli, C. M. Epstein, and W. H. Thompson. Department of Psychology, Stanford University, Stanford, CA 94305; Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892.

Picture-naming (PN) priming has been shown to be mediated by perceptual and conceptual processes. Priming produced by repeated picture naming (PNP) involves perceptual and conceptual processes; priming produced by prior reading of picture labels (words) (WRP) involves knowledge-based conceptual processes. The neural anatomical bases for the perceptual and conceptual processes underlying PN priming were examined using post-mortem anatomy, by performing a PN task, regional cerebral blood flow was measured in 12 normal subjects tested in a Scanditronix II scanner using six injections of 15O. The design employed 3 study-test conditions that were administered during each test phase. In the baseline condition, subjects named pictures and then different, nonstudied pictures. In the WRP condition, subjects named pictures and then named the same pictures. In the WRP and WRP naming were rated as more activation in right precentral, left superior frontal gyrus, right middle frontal gyrus, and cingulate for PN priming, and more activation in left hippocampus, left temporal gyrus, left inferior frontal gyrus, and left occipital lobe for WRP naming. Across comparisons, perceptual processes tended to involve bilateral posterior regions of the brain, whereas conceptual processes engaged more medial temporal and anterior regions. These results suggest that separable neural substrates mediate perceptual and conceptual priming processes.


It has been postulated that during learning an interaction is established between the medial temporal memory system and distributed storage sites in the neocortex. Using functional MRI (fMRI), we studied young normal subjects while they performed picture encoding (n = 25) and picture recognition (n = 15) tasks. High speed echo-planar imaging (EPI) techniques were combined with asymmetric spin-echo imaging sequences to simultaneously evaluate fMRI signal changes throughout the whole brain. To assess picture encoding, subjects were asked to look at a consecutive series of complex, colored pictures so that they could recognize them later. As a control condition, subjects were scanned while viewing a single repeating picture. Subtraction (encoding minus control) showed significant signal intensity increases bilaterally in hippocampal and parahippocampal regions and at the temporal-occipital junction. Recognition memory was assessed by subtracting images obtained during a two-alternative, forced-choice identification test (identify previously viewed pictures) from images obtained during a two-alternative, forced-choice identification test (identify indoor vs. outdoor pictures). The subtraction images revealed significant (P<0.01) differences bilaterally in prefrontal cortex (areas 46 and 10). This fMRI experiment provides new evidence about the participation of the medial temporal system during information encoding and also about the locus of cortical areas that support picture recognition memory.

530.9 HIPPOCAMPAL ACTIVATION IN DMI EVOKE BY DEMAND FOR DECLARATIVE MEMORY: A ROLE FOR EMBODISED BOUNDARY STREAMS OF INFORMATION. N. J. Cohen*, C. Barany, X. Hu. T. V. Tomes, E. T. K. U. Unglesschak, Beckwith Institute & Department of Psychology, University of Illinois, Urbana-Champaign, IL, 61801; Center for Magnetic Resonance Research, University of Minnesota Medical School, Minneapolis, MN, 55455.

Despite abundant evidence linking the hippocampal system to memory, it has been difficult to find reliable hippocampal activation in functional brain imaging studies of declarative memory. Here we report robust hippocampal activation in a functional MRI imaging (fMRI) study that was based on Cohen and Eichenbaum's proposal linking hippocampal system function to declarative memory for the relations among perceptually distinct objects. Seven normal volunteers participated in two tasks involving the processing of color images of faces. During baseline conditions, the faces were presented side-by-side, or with the superimposition of a name, or of a name and an occupation-related icon. In the baseline task, subjects had to make decisions about each face, ignoring any names or icons that were presented with them. In the memory task, subjects had a series of alternating study and test conditions requiring the binding together of the 3 different declarative-memory dependent details. In each of the 7 S's, activation (increased signal intensity on 99%-confidence T-maps) was observed in left hippocampal and parahippocampal regions in a comparison of baseline task versus memory task. Activation was initiated at the onset of the study phase of the memory task condition and stayed elevated above baseline levels throughout the 8 memory (study & test) task conditions. It appears that the same LH region that was important for semantic encoding was also the locus for item-specific semantic memory for these words. These results indicate that MRI may be useful in detecting the brain locus of semantic encoding and memory. Supported by grants from the NIMH (15T096926), the Alzheimer's Association, Stanford Office of Technology and Licensing, and the Lucas Foundation.


Brain imaging (PET) and fMRI have been used to reveal a role for the left inferior frontal gyrus (LIFG) in the retrieval of well-learned verbal material, including regionally specific activation that is modulated by attentional manipulations of the task. In the present study, we hoped to investigate the role of the LIFG in the initial encoding of semantic information. As before, 13 normal volunteer subjects were scanned using a combination of fMRI and PET. Imaging was performed in a 1.5T scanner (T2* flip angles23°). Two 7-mm functional slices were acquired separately in the Talairach & Tournois (1988) coronal plane (x=-32, y=-39, 1.8mm pixel size). Images were acquired twice every 1.5 seconds for 5.5 minutes. Subjects viewed words continuously and switched between performing semantic judgements (e.g., respond to abstract but not concrete words) and nonsemantic judgements (e.g., respond to words printed in upper but not lower case) every 20 seconds. Images were analyzed by correlating activation over time to a sinusoidal reference vector. Areas in the LIFG, but not in the analogous region in the right hemisphere, showed activation that correlated significantly with the reference vector (r<.30). There was an increase in activation during the semantic task. In a second experiment at the same slices, subjects performed the semantic task on blocks of 20 words shown for a first time (new) and then repeated for a second time (old) in successive blocks. There was less activation in the LIFG for old than for new words. The deactivation may reflect implicit conceptual memory (repetition priming) for old words. Thus, it appears that the same LIFG region that was important for semantic encoding was also the locus for item-specific semantic memory for these words. These results indicate that fMRI may be useful in detecting the brain locus of semantic encoding and memory. Supported by grants from the NIH (PINS09628), the Alzheimer's Association, Stanford Office of Technology and Licensing, and the Lucas Foundation.

530.11 VERBAL MEMORY IMPAIRMENT AFTER RIGHT TEMPORAL-LOBE EXCISONS: FOLLOW-UP STUDY. PIERSERIO, A. Cupani, P. D. Bogliolo, F. Vaghi-Schena, SPONI-European Brain and Behaviour Society, Neuroscience Unit, Institute of Child Health, University of London, Mecklenburg Square London WC1N 2APT.UK.

Verbal memory following left temporal lobectomy for epilepsy was assessed in relation to both sides of the hemisphere. The patients were compared to a group of patients with right temporal lobectomy who were matched for age, sex, language lateralization, and with the normal control group. The left temporal lobectomy group showed more impairment in verbal memory across the tests. This was true for both the verbal memory tasks and the tests of reading comprehension. The results of this study are consistent with the hypothesis that there is a more extensive and more severe deficit in verbal memory following left temporal lobectomy than following right temporal lobectomy.

530.12 A POSTERION EMISION TOMOGRAPHY (PET) STUDY OF REGIONAL CEREBRAL DURING FLOW RECORDING OF THE VERBAL MEMORY. D.S. O'Leary, N. Andreasen, R.R. Hurtig, Mental Health Clinical Research Center, Dept of Psychiatry, Univ of Iowa College of Medicine, Iowa City IA 52242.

We assessed CBP with PET using the bolus 15O-labelled water method in 13 normal adult volunteers who were tested twice: once 1 week prior to PET imaging subjects learned a 15 word list and a short verbal passage. Subjects practiced the material to 100% correct recall again one day before imaging. During the acquisition, the repetition PET study the list and passage were read to the subjects immediately prior to image acquisition. During two other conditions a novel word list and novel passage were read to the subjects. Subsequent to the each condition each subject immediately began recall; this was timed for each condition to occur about 10 seconds prior to arrival of the bolus (15O water). Regional cerebral metabolic rates for glucose were measured in the study were a resting baseline, recall of a personal episode from the subject's past, a verbal fluency task, and a repeat of the familiar verbal passage to assess replicability of metabolism. Results showed that metabolism was elevated in the right prefrontal cortex in the subject's past, a verbal fluency task, and a repeat of the familiar verbal passage to assess replicability of metabolism. Results showed that metabolism was elevated in the right prefrontal cortex. Recognition memory was assessed by subtracting images obtained during a two-alternative, forced-choice identification test (identify indoor or outdoor pictures). The subtraction images revealed significant differences bilaterally in prefrontal cortex (areas 46 and 10). This fMRI experiment provides new evidence about the participation of the medial temporal system during information encoding and also about the locus of cortical areas that support picture recognition memory.

530.13 SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
process outgrowth and adhesion molecules

S31.1 DEVELOPMENTAL INCREASE IN POLYSACCHARIDE ON NCAM FACILITATES AXONAL OUTGROWTH FROM CORTICOSPINAL AXONS. M.A. Daston, R., B. Baumgarten, 1, U. Rutishauser, 2 and D.D.M. O'Leary. 1 1. The Salk Institute and 2 Case Western Reserve University.

Corticospinal tract (CST) axons intercept their spinal cord targets by extending collateral branches. These branches begin to arise along the axon on P4, but the primary growth cones have passed their targets. The neural cell adhesion molecule (NCAM) is often modified by the addition of polysaccharide (PSA). PSA has been implicated as a regulator of cell shape and adhesion of its ability to alter cell-cell interactions. We have found that the extension of collateral branches from CST axons is influenced by the PSA moiety on NCAM.

To assess the distribution of NCAM-PSA in the CST, we immunostained transverse sections of rat spinal cords over the first postnatal week. At all ages, NCAM is broadly distributed in the spinal gray, found in the spinal gray terminal fields and in the CST. However, PSA staining is weak or absent over most of the spinal cord, with the exception of the CST. The PSA staining is in the CST and is weak and patchy on P2, but becomes intense by P4, the age when collateral branches begin to form.

To determine the role of PSA in the extension of collateral branches, we injected endo N, which cleaves PSA from NCAM, into the fourth ventricle of neonatal rats. Immunostaining revealed that PSA was completely eliminated whereas NCAM appeared normal. CST axons were labeled by Dil injected in motor cortex. In endo N-treated rats examined at P4, 5 or 6, many fewer axon collaterals extended from the CST into the surrounding spinal gray compared to normal rats or rats injected with inactive endo N. These results suggest that the increase in PSA that occurs preferentially in the CST facilitates innervation of the spinal gray. The role of PSA may be to attenuate axon-axon interactions in the CST and thus enhance an axon's ability to respond to targeting cues.

S31.3 REGIONAL LASER INACTIVATION OF L1 AND NCAM AFFECTS GROWTH CONE MOTILITY IN CHICK DRG NEURONS. K. Takeda * T. Chan and D. Q. Levy. Dept. of Cell and Mol. Biology, Harvard University, Cambridge, MA 02138. L1 and NCAM are neural cell adhesion molecules that belong to the Ig superfamily and are abundantly expressed in the developing nervous system. We have used regional inactivation of these molecules to investigate the role they play in growth cone motility. Microscale laser inactivation, using a哪怕 well-targeting laser irradiation (micro-CALI) was performed on chick DRG neurons cultured on laminin and L1 substrates. Using micro-CALI, we are able to produce highly localized damage of target molecules in the growth cone that are bound with malachite green (MG)-labeling antibodies. To inactivate intracellular domains of the molecules, the MG-labeled antibodies were loaded with photobleaching by irradiation; a high level of retention of the antibodies was observed up to 8 h later by immunocytochemistry. Micro-CALI of L1 and NCAM (180x3k) in the growth cone of DRG neurons treated with free Gfobiosyl and lamellipodium collapse. When DRG neurons were grown on L1 substrate, micro-CALI of L1 resulted in dramatic retraction of the whole neuron as well as intracellular organelles. Laser irradiation of neurons loaded with MG-labeled bovine serum albumin, MG-labeled non-specific antibodies or without antibodies did not affect growth cone motility. These data strongly suggest that L1 and NCAM play a key role in growth cone motility. Our findings may help us to define the physiological role of L1 and NCAM in axonal pathfinding mechanisms.


Amacrine cells are retinal interneurons that project relatively short processes onto neighboring cells exclusively within the inner plexiform layer (IPL). Our studies have focused on defining the factors that regulate amacrine cell neurite growth. Recently, we demonstrated that NCAM functions as a neurite-growth-promoting factor for rat amacrine cells in culture. However, NCAM is found in a number of different forms which can alter its function, one of which involves a decrease in the polysialic (PSA) content as the nervous system matures. We have found similar decreases in PSA levels within the IPL. At postnatal day 3 (P3) in the rat retina the IPL is highly immunoreactive for PSA, whereas at later ages (P25) PSA immunoreactivity is absent. The loss of PSA has been shown to enhance NCAM-NCAM adhesion and inhibit NCAM-dependent neurite growth in other cell types. We tested the effectiveness of NCAM to promote neurite growth from amacrine cells in culture after PSA was removed. Amacrine cells derived from P3 rat retina were cultured on affinity purified NCAM rich in PSA. PSA was removed from the culture substrates by using BSA and Triton X-100. After 24 hours in culture, cultured neurites increased by 95% when PSA was removed from the culture substrates and 78% when PSA was removed from both the culture substrate and amacrine cells surfaces using the enzyme Endo-N. Total neurite lengths for amacrine cells after 24 hours in culture increased by 9% when PSA was removed from the substrate and 78% when PSA was removed from both the culture substrate and amacrine cells surfaces (Control, 67±10.9; Neuraminidase, 64±7.8; Endo-N, 110±44). After 8 hours in culture, 86±2.1% of the amacrine cells had formed distinct lamellipodia following PSA removal whereas only 84±1.4% did in control cultures. These results clearly show an increase in neurite growth from amacrine cells cultured on purified NCAM substrates. Supp. by NIH NS 31365 and the Albert Sloman Association.
FUNCTIONAL ANALYSIS OF CELL ADHESION MOLECULE L1 USING DEFECTIVE HERPES SIMPLEX VIRUS VECTORS. T. Yezzi, Jr., R. Martuza, G. D. Rabinovitch. Dept. of Neurosurgery, Georgetown University Medical Center, Washington, D.C. 20007.

L1, a member of immunoglobulin superfamily, is a transmembrane glycoprotein that promotes neurite outgrowth and cell migration. L1 is expressed in neurons and peripheral Schwann cells and potentially involved in neural regeneration through a homophilic adhesion mechanism. Plasmid-based defective herpes simplex virus (HSV) vectors are an efficient means to transfer genes into the central nervous system (CNS). To characterize the functional role of L1, we constructed several defective HSV vectors containing the CMV or GFAP promoter upstream of human or rat L1 DNA.

Primary, cultured rat astrocytes and several cell lines, which do not express L1, were infected with defective HSV vectors containing human or rat L1. At 30 hours after infection, cells were fixed and stained with polyclonal antibody against L1. The CMV promoter efficiently drove L1 expression in both primary astrocytes and cell lines, whereas GFAP promoter drove L1 specifically in primary astrocytes. Primary astrocytes, Vero cells and rabbit skin cells expressing L1, after infection with defective HSV vectors, were morphologically altered. The cell lines contained long processes with many branches. The morphological change was not observed in cells infected with defective HSV vectors expressing α-galactosidase or alkaline phosphatase.

These defective HSV vectors will be used to study the functional role of L1 in neural regeneration.


We examined the effect of substrate-bound L1 on the development of polarity by embryonic rat hippocampal neurons in culture. Neurons were cultured on immunostreptavidin-coated glass coverslips coated with 8D9, the chick homolog of L1, or on coverslips coated with polylactic acid (PL). L1 selectively stimulated axonal growth and accelerated the development of morphological polarity. After 8 h in culture, 37% of cells grown on L1 had formed axons compared with less than 1% of cells on PL. At 24 h, 71% of cells on PL had formed axons, and the axons of cells grown on L1 were about four times longer than those on PL. In addition, 24% of cells on L1 developed two axons after 24 h. The length of microprocesses did not differ on the two substrates. Despite these pronounced changes in cell shape, polarity markers segregated similarly on the two substrates. L1 became restricted to axons while MAP2, transferrin receptor, and LDL receptor-related protein segregated to axons and dendrites on both L1 and PL. By 3 weeks in culture, cells grown on L1 had formed well-differentiated dendrites, as indicated by their morphology after MAP2 staining, and were contaminated by numerous synaptophysin-positive presynaptic terminals. The selective effect of L1 on axonal growth is not surprising, since substrate-bound L1 would be expected to bind homophilically to neuronal L1, which is restricted to the axonal surface. The relatively normal development of dendrites on the L1 substrates may have been mediated by small amounts of L1 on the dendrites. Alternatively, an adhesion molecule that interacts with L1 in a heterophilic manner may be present on dendrites. Finally, other proteins secreted by the cultured cells or present in the medium may bind to the nitrocellulose and permit dendritic growth.

This work was supported by NIH grant NS17112, NEI 05285 (V.L.) and by a graduate research fellowship from the NSF.


Toward the goal of engineering live neuronal networks, we have begun a search for culture substrates that are selectively adhesive for specific cell types and their neurites, either in axons or dendrites. This investigation is possible because of the development of a serum-free medium for growth of isolated neurons without glia (B2/Neurobasal, GIBCO; Brewer et al., 1993). J. Neurosci. Res. 35-576). Glass substrates were coated with either poly-D-lysine (PL) or PL followed by laminin, or pleiotrophin. Neurons from embryonic day 18 rat hippocampus were plated at 80 cells/mm². Survival (live/total cells) was equivalent on the three substrates. After 1 day, neurotogenesis was dramatically enhanced on laminin or pleiotrophin, compared to polylysine. After 4 days, the uniform narrow processes on pleiotrophin had continued to grow, while those on laminin largely stopped. In contrast, cells on PL grew shorter tapered processes, characteristic of dendrites. The shorter tapered processes reacted with antibodies recognizing the cytoskeletal axonal marker tau. By selective patterning of substrates, it may be possible to specifically direct axonal and dendritic growth.


Neurofilaments are neuronal intermediate filaments comprised of three polypeptide subunits NF-L, NF-M and NF-H. Increasing evidence has shown that these subunits are required for the development of normal axonal caliber, a crucial determinant of axonal conduction velocity. To test the role of each individual subunit, we have over-expressed different subunits in transgenic mice. Surprisingly, a simple increase in number of filaments by a 2-fold increase in NF-L inhibits radial axonal growth, producing smaller axons. An increase (by 2 fold) in either NF-H, NF-M or both, (presumably increasing the side arms), inhibits radial axonal growth to an even larger extent. However, only an increase of NF-L with either NF-M or NF-H produces larger axons. These data demonstrate that radial axonal growth requires not only an appropriate number of filaments but also filaments comprised of a balanced composition of the different subunits and in particular, a normal ratio between the content of NF-L and either of two large subunits, NF-M and NF-H.

MAP2 EXPRESSION AND NEURITIC BRANCHING ARE REDUCED WHEN SPINAL CORD NEURONS ARE CO-CULTURED WITH WOBBLE MOUSE ASTROCYTES. D. Hata-Antчрежecz, M. Ano, Y. Nakamura, E. Rieger, 1, 1 INSERM, Unité 135, 17 rue du Faur-Muelin, Paris 75005; 2Université Paris VI, 6 place Jussieu, Paris 75005.

Embryonic normal mouse neurons can be cultured on layers of primary astrocyte culture and are identified by microtubule-associated proteins, MAP2, exhibiting a high degree of neuronal cell type specificity. With the aid of specific antibodies to MAP2, we studied the neurite outgrowth and examined by quantitative image analysis the changes in MAP2 staining which allows a direct visualization of neurite outgrowth. We performed cocultures of embryonic normal neurons with both normal and wobbler astrocytes. These mutant astrocytes are strongly GFAP-reactive and do not develop a neurite and in vitro, morphological modifications in process outgrowth and arrangement (J. Neurocytol., 22, 179-192, 1994). We found that the number of MAP2 negative neurites coupled to wobbler astrocytes was much higher than those to normal astrocytes, pointing towards a defect in neurite outgrowth mediated by wobbler astrocytes. We thus studied the expression and cell distribution of MAP2 in both conditions. Quantitative image analysis showed a reduced number of neurites and neurite branching points in the wobbler astrocyte-neuron coupled cultures compared to the normal cocultures. These results are in agreement with the recent observation of Wolfe and Vasse-Callowley (1991) that in vivo the extent and branching of neurite arborization is abnormal in wobbler mice. Similar observations were made when normal neurons and astrocytes were cocultured in the presence of conditioned medium against astrocyte outgrowth. Altogether, these data strongly support the hypothesis that neurite outgrowth is inhibited under the influence of soluble factors secreted by wobbler astrocytes and not normal astrocytes. These observations suggest that wobbler astrocytes possibly produce high amounts of glia-derived growth inhibitors.
532.3

CHARACTERIZATION AND REASSEMBLY OF ALPHA-
INTERNEXIN (NF66) ISOMORPHS FROM BOVINE SPINAL
CORD, M.E. Miller* and B.J. Ballin, Med. Coll. of PA, Dept.
of Pathology and Lab Med., 3300 Henry Ave., Phila., PA 19129.

We have reassembled alpha-internexin (NF66), a member of the type IV family of intermediate filaments, can be
isolated from bovine spinal cord and that purified NF66 is capable of reassembling (as well as coassembling with other type IV intermediate filaments) into ~10-nm-diameter filaments (Ballin & Miller (1994) J. Neurosci., Res., in press). During purification, a high molecular weight isoform of NF66 was found to co-elute with the high molecular weight neurofilament subunit (NF-H) following anion exchange chromatography of an enriched cytoskeletal fraction. These two proteins were found to be inseparable by gel filtration and a gel elution strategy (Miller et al. (1994) J. Neurosci. Meth., submitted) to use purified NF66 to homogeneity for reassemblability studies. We now demonstrate the existence of lower molecular weight isoforms (<66kD) of bovine NF66 that can be isolated by successive cation and anion exchange chromatography. These isoforms of NF66 are also shown to be capable of reassembling into ~10-nm-diameter filaments by negative staining and immunoelectron microscopy. Characterization of these NF66 isoforms by 1-2-D gel electrophoresis and Western blotting suggests that these polypeptides are NF66 phosphoisoforms. Supported by PHS grant AG10160.

532.4

ALTERING THE PROTEIN COMPOSITION OF DEVELOPING AXONS:
MICROINJECTION OF A NEUROFILAMENT ANTIBODY TO STUDY NEURITE

The cytoskeletons of newly differentiating Xenopus laevis spinal cord axons contain a middle- (NF-M) and a lower molecular weight neurofilament, proto NF-M. Microinjection of antibodies against NF-M into embryonic blastomeres resulted in restricted expression of NF-M immunoreactivity within the neurons of intact embryo and inhibits peripheral nerve development (J. Comp. Neurol. 308:157). To better understand these effects we examined the consequences of anti-neurofilament antibody injection on embryonic spinal cord isolated in culture. Single blastomeres of 2-cell stage embryo were microinjected with an antibody that reacts with FITC-
cones. Injections at the 2-cell stage inoculated only half of the neurons, those being a probe to the injected neurons, which were identified by FITC-fluorescence, to normal ones within the same culture. After injection, neural tubes were dissociated and cultured at stage 22. Immunocytochemistry with rhodamine-labeled secondary antibodies confirmed that for at least the first 24 hrs in culture, the injected antibody was uniformly distributed throughout soma and within neurites. Furthermore, the neurofilament antibody successfully altered the normal distribution of neurofilaments within neurons. For example, neurites of uninjected neurons are evenly filled with NF-M-
immunoreactivity. In contrast, in injected neurons, NF-M accumulated within the same as leaving neurites devoid of detectable neurofilaments. These results resemble the effects observed in intact embryos. Next, we will measure the rates of axonal outgrowth in the absence of neurofilaments. Supported by NIH R29-NS30682.

533.5

OVER-EXPRESSED DREBRIEN FORMED THICK, CURVING
BUNDLES OF ACTIN FILAMENTS, FROM WHICH TROPOMYOSIN

Drebrin is an actin-binding protein rich in the neurite. During the development of the brain, it is localized at the submembranous region of migrating fibroblasts and growing axons and dendrites. By transfection of fibroblasts with drebrin cDNA, the outgrowth of highly branched, neurite-like cell processes is induced from the cells that the protein is expressed at high levels. We demonstrate by an immunocytochemical method that the drebrin expressed in transfected cells binds to stress fibers and, as a consequence, thick, curving bundles of actin filaments are formed. Such bundles were observed not only in the highly branched, neurite-like cell processes but also in the cell bodies of transfectedants. Tropomyosin was dissociated from actin filaments in these cells. Biochemical analysis also revealed that drebrin strongly inhibited the actin-binding activity of tropomyosin by competitive binding. These data suggest that actin filaments that bind drebrin form a novel class of actin filaments, which may play a role in neuronal morphogenesis.

533.6

DISTRIBUTION OF CYTOSKELETAL PROTEINS IN GROWTH CONES IS
INFLUENCED BY CONTACT WITH SUBSTRATE-BOUND L1, N-CADHERIN
AND LAMININ. G.M. Ritchie, C.J. Little, J. Lampe, Dept. of Neurobiol. Stores, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

We have previously shown that retinal ganglion cell (RGC) growth cones exhibit characteristic morphologies dependent upon the substrate on which they are grown (Payne, et al. (1992) Cell Motil. Cytoskel. 21:65-73). Upon contact with substrates, the growth cones display dynamic changes in morphology (Burden, et al. (1992) Soc. Neurosci. Abst. 814.10) that may be due to extensive restructuring of the cytoskeleton. In this study, we used immunocytochemistry to examine the distribution of three cytoskeletal elements in RGC growth cones growing on L1, n-cadherin or laminin individually, as well as on plates coated with alternating layers of these substrates.

We have observed distinct staining patterns for f-actin, microtubules (MTs) and neurofilaments (NFs) in growth cones growing on individual substrates. At border regions between two substrates, growth cones that established lamellipodial contact with the new substrate were observed to have f-actin and MT staining patterns appropriate for the newly encountered substrate. Contact via filopodia alone did not evoke this change. Since the majority of the growth cone had crossed onto the second substrate, redistribution of NFs was also observed. These results suggest that the major cytoskeletal elements of growth cones are rapidly restructured in response to substrate contact. Such changes are likely to be crucial for growth cone guidance. Supported by NEI grant 05285.

533.7

LOCALIZED RE-EXPRESSION OF A DEVELOPMENTALLY-REGULATED
FORM OF MAP 1B DURING AXONAL REGENERATION IN VITRO. D.A. Trape, J.P. Goldman and P.R. Gordon-Weeks*. Biomedical Sciences Division, King's College, Sand, London WC2R 2LS, U.K.

Microtubule organization in growth cones is known to be essential for neurite extension. Phosphorylation of microtubule-associated proteins (MAPs) modifies their affinity for microtubules and this might affect neurite growth. Monoclonal antibody 150 (mab 150) recognizes a novel phosphorylation epitope on MAP 1B which is transiently expressed during development of the nervous system (Eur. J. Neurosci. 1:1902-1911). In the present study, expression of this form of MAP 1B was studied during axonal regeneration in vitro.

Short lengths of spinal nerve with their attached dorsal root ganglia were removed from adult mice, explaned into Matrigel and maintained in serum-free medium for up to 8 days. Profuse growth of naked axons occurred within one day from the cut ends of both the peripheral nerve and the dorsal root, and continued throughout the experimental period. Some axons within the same nerve whilst others showed prominent varicosities. Immunocytochemical staining using mab 150 was observed along the whole length of the axons growing out of the explant, but not within the nerve itself. The stained extended to the growth cones which had elaborate morphology. Other antibodies (e.g. to OAP-43) labeled axons within the nerve as well as those growing in Matrigel. In preparations where the peripheral nerve had been dissected away, mab 150 staining was absent from the nerve proximal to the crush site, but present in axons which had regenerated within the nerve distal to the crush. These results indicate that re-
expression, during axonal regeneration of the form of MAP 1B recognized by mab 150, is restricted to the newly formed segments of axons. The correlation between its expression and axonal growth during development and regeneration suggests that it may play a role in axonal extension. (Supported by MRC).

533.8

REORGANIZATION OF MICROTUBULES IN CORTICAL GROWTH CONES
DURING CELL-CELL INTERACTIONS. M. Lin* and K. Kallil. Neuroscience Training Program and Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

Growth cone behaviors such as advance, retraction, fasciculation, turning, and branching are accomplished by reorganization of the microtubule cytoskeleton. To understand how microtubules contribute to behaviors of CNS neuronal growth cones, we have studied the disposition of microtubules in growth cones of embryonic cortical neurons as they interacted with neighboring cells. We have used high resolution video enhanced Nomarski optics to visualize morphologies of fixed growth cones captured during dynamic growth cone fluorescence images of the same growth cones stained immunocytochemically with anti beta tubulin were used to locate the positions of microtubules during these behaviors. We found that microtubules were symmetrically distributed (NEF), proximal region of growth cones advancing in straight trajectories. In contrast, growth cones turning away from straight trajectories had microtubules oriented in the direction of turning. When growth cones were fasciculated with other neurites the orientation of their microtubules became aligned with those of the neurites. Microtubules also invaded the branches along neurites as well as growing neurites. In video microscopy, we have previously observed that when growth cones collapse and withdrew they often leave behind a long filopodial strand upon which the growth cone restructures. Immunocytochemistry revealed that these strands were usually invaded by microtubules. Preliminary results in which microtubules were stained with antibodies to detyrosinated tubulin suggest that the microtubules observed during dynamic growth cone behaviors are relatively labile. These results suggest that the invasion of labile microtubules may underlie growth cone behaviors that require the growth cone to advance...

Drebrin are developmentally regulated actin-binding proteins. Although neurite outgrowth is the first step in neuronal network formation, the molecular mechanism of neurite outgrowth is not yet clear. To determine the role of drebrin in these phenomena, we have studied the rat neuroblastoma cell line, B104, which constitutively expresses drebrin. Deprivation of serum or retinoic acid treatment induces a characteristic change in morphology; cells produce drebrin-positive processes. Then, in an effort to interfere with the expression of drebrin in B104 cells, we transfected them with an antisense construct of human drebrin E cDNA (Biochem. Biophys. Res. Comm. 190: 468-472, 1993), driven by a β-actin promoter. Two stable antisense cell lines from separate transfections were isolated and shown to be drebrin-negative by Western blot analysis and immunofluorescence studies. The antisense cell lines were inhibited in their ability to extend significant neurite processes in response to serum deprivation or retinoic acid treatment. These data support the conclusion that the actin-binding protein, drebrin, is required for the formation of stable neurite processes.

MECHANISMS OF AXON GUIDANCE

532.1

LAZARILLO: A GPI-LINKED GLYCOPROTEIN INVOLVED IN AXON GUIDANCE IN THE STRIATAL GRANULAR網路IS A MEMBER OF THE LIPOCALIN FAMILY. D. Sánchez, M.D. Gaetano, and M.J. Bastiani* 1Department of Biology, University of Utah, Salt Lake City, Utah 84112, U.S.A.

Our work is to determine G-protein-coupled receptors that ensure neurites find their appropriate pathway to target cells during neurogenesis. We are studying a new cell surface molecule called Lazarillo which is expressed by a subset of neurons and neuronal precursors in the grasshopper nervous system. This molecule is linked to the plasma membrane by a glycosylphosphatidylinositol (GPI) group and posttranslational N-linked oligosaccharides (native Mr=533.4 kD, deglycosylated Mr=28 kD). The monoclonal antibody against Lazarillo (10E6 MAbs) is able to block the directed growth of neurites in cultured embryos. In particular, a pair of commissural pioneer neurons are disrupted; their growth cones are not arrested but merely change their direction of growth.

We have identified and characterized a cDNA clone coding for the Lazarillo protein. The predicted protein sequence has the signal peptide necessary to target the protein to the endoplasmic reticulum and the GPI anchor to the membrane by the GPI tail. It possesses seven N-linked glycosylation sites and four cysteine residues. The cDNA has a long (2.3 kb) 3′ untranslated region. In situ hybridization using the whole cDNA sequence as probe shows that Lazarillo mRNA co-localizes with the 10E6 MAbs. Sequence comparison reveals that Lazarillo belongs to the gigaxonin family of extracellular carriers of small hydrophobic ligands in a wide variety of systems. A phylogenetic analysis of the family sets Lazarillo in a cluster containing the mammalian Apolipoprotein D, the insect binil binding proteins, and all the serum retinol binding proteins from vertebrates. Lazarillo is, to the best of our knowledge, the first lipocalin (1 GPI-anchored to the plasma membrane of cells), (2) restricted to a subset of neurons and neuroblasts in a developing nervous system, and (3) involved in growth cone outgrowth. Supported by NIH (NS 20387), Fulbright (M.D.G.) and Fogarty (D.S.) fellowships, M.D.G. and D.S. contributed equally in this work.

532.2

CALCINEURIN ENTRY AND CALCIUM STORES CONTROL NERVE GROWTH IN AN APPLIED ELECTRIC FIELD. B. Stewart & C.D. McCelland 2Department of Biomedical Sciences, University of Aberdeen, Aberdeen AB9 1AS, Scotland.

Cultured neurons show striking growth responses to a small dc applied electric field of physiological magnitude. The Calcium entry hypothesis has been implicated. Neurites from Stage 20 Xenopus neural tubes grown on tissue culture plastic in a dc field of 150mV/mm, 1. grow faster than in the absence of the field; 2. grow faster cathodally than anodally; and 3. turn towards the cathode. Using a pharmacological approach, we have studied both the role of selective voltage dependent calcium channels (VDCCs) and of calcium release from intracellular stores in the control of these three responses. We report 1. that faster growth in an applied electric field is lost in the presence of the P-type VDCC inhibitor omega-agatoxin IVA and in the presence of thapsigargin, which disrupts calcium release from inositoltrisphosphate sensitive intracellular stores; 2. that cyanide, which disrupts calcium-induced calcium release from intracellular stores, abolishes the growth rate differential between cathode and anode facing neurites and 3. that the P- and N-type VDCC inhibitors omega-agatoxin IVA and omega-conotoxin GVIA respectively, as well as cyanide and thapsigargin each partially inhibit cathodal orientation. We conclude that calcium entry through specific VDCCs in the growth cone, linked to calcium release from specific intracellular stores controls different aspects of electric field-induced growth and orientation.

532.3


Recent experiments suggest important roles for sulfated proteoglycans (PGs) in influencing axonal growth. In addition, chondroitin sulfate and keratan sulfate PG (CSPG, KS PG) expression patterns correlate at certain times with nerve growth across the developing retina, such as the corneal subplate, and at other times with regions which axons distinctly avoid, such as the midline of the superior colliculus. We have examined the expression of PGs in the superior colliculus of developing hamster, around the time of retinal axon ingrowth and arborization in this target. PGs were isolated from tectal homogenates by anion exchange chromatography, radiolabelled, and digested with enzymes to remove specific glycosaminoglycan (GAG) chains. Resulting protein cores were separated by electrophoresis. During the first postnatal week, we have identified PGs, which, on the basis of molecular weight, solubility properties, and GAG type, bear distinct resemblance to developmentally regulated PGs that are previously isolated from rat brain. Early postnatal tectal contained at least four distinct HSPGs, with molecular weights corresponding to glypicans, cerebrotenyl, and M410 (see Hamond and Lande, Nature 4, 1990). CSPGs of sizes consistent with neurocan, S2 and M8 were also identified. In addition, two novel bands appeared in response to digestion with keratanase, although the possibility of proteolytic contamination in the keratanase has not yet been ruled out. Experiments are currently under way to isolate PGs at other ages, and to localize their sites of expression within the superior colliculus, with emphasis on gaining potential insights from comparing expression along the midline and in retinoreceptive zones of lateral regions. Supported by NIH grants EY05054, NS26682, and EY02621.

532.4

TWO CHONDROTIN SULFATE PROTEOGLYCANS EXPRESSED DURING THE SEGMENTATION OF THE PERIPHERAL NERVOUS SYSTEM OF THE CHICK. C. Ring and W. Hallberg, Univ. of Pennsylvania School of Medicine, Dept. of Neurobiology, 15261.

Chondroitin sulfate proteoglycans (CSPGs) purified from Carnegie have been shown to be inhibitory to neurite outgrowth in vivo. Based on these findings, it has been hypothesized that CSPGs function as barrier molecules to neurite outgrowth in vivo. We wanted to examine if CSPGs derived from cartilaginous embryonic tissues would have similar effects. We have isolated two monoclonal antibodies (Mabs), 9BA12 and 9BA14, which are specific for CSPGs that are developmentally expressed in the trunk region of the chick embryo. The 239 kDa BM has been shown to recognize collagen type IX proteoglycan (CIXPG). It is expressed in the posterior segment of the somite in the time period of the somite anlage project, and neural crest cells migrate through the anterior sclerotome. Dorsal root ganglion (DRG) explants can adhere to, and extend neurites on, micrometre-bound CIXPG. Neurites from DRG explants grown on a combination of fibronectin/CIXPG appear shorter and more fasciculated than control explants grown on fibronectin alone. The 9BA12 mAb recognizes an as yet unidentified CSPG in neural tissue. It is expressed throughout the trunk region of the developing chick embryo, in the neural tube, throughout the somite, and is absent only from the dermamyotome. As with the CIXPG, DRG explants can adhere to and extend neurites on, micrometre-bound 9BA12 antigens. Alternately, in combination with fibronectin, the explants were able to extend neurites on the 9BA12 antigens that appeared identical to control explants grown on fibronectin alone. Our study demonstrates that CIXPG is the posterior sclerotome may contribute to the avoidance of this tissue by developing neurites and migrating neural crest cells. In addition, we conclude that not all CSPGs are inhibitory to axon outgrowth, and that careful attention be paid to the source and type of proteoglycan used in neurite outgrowth assays.
533.5 LAMININ AND FIBRONECTIN GUIDEPOSTS SIGNAL SUSAINED BUT OPPOSITE EFFECTS ON PASSING GROWTH CONES. T. B. Kahn, M. F. Schmidtk and D. B. Kaper*. Dep. of Anatomy and Neurobiology, OVCN2523; S Division of Biology, Caltech, Pasadena, CA 91125.

Guidepost cells affect behavior and navigation of growing cones in vivo. Yet, the nature of communication and the type of signals employed between growing cones and guideposts are largely undefined. Here we report that in a vitro assay system both behavior and navigation of advancing DRG growth cones of the chick were significantly altered by transient encounters with monomolecular model-guideposts: 4.5μm polystyrene beads covalently coated with potential guidance molecules. Evoked growth cone behavior, a series of stereotypy responses, depended on the molecular nature of the model guidepost. Growth cones most often paused and displayed filopodial sampling at guideposts. Laminin-model guideposts caused a sustained, two-fold increase in growth cone extension. Fibronectin-substrate (p<0.01; n=17) while fibronectin-model guideposts led to a sustained, two-fold decrease of the rate of advance on a laminin substrate (p<0.01; n=13). Notably, these effects lasted substantially beyond the time of physical contact between growth cone and model guidepost. Arrays of laminin-model guideposts produced a satellite growth cone advance and provided unambiguous, directional guidance. Interestingly, an increased overall growth rate (above that on the polystyrene substrate) was observed equivalent to a homogenous laminin substrate, despite periodic pausing occurring at each guidepost. Taken together, these results demonstrate that 1) as little as an individual molecular species is required to designate a point in space as a functional guidepost, 2) transient growth cone-guidepost contacts appear to initiate signalling pathways rather than just influence adhesion, and 3) the enforced pausing and sampling may represent a unique feature of guidepost-mediated pathfinding resulting in a periodic reassembly of the environment by the growth cones.

533.7 THE CLONING OF A CHICK BRAIN cDNA ENCODING A PROTEIN SEQUENCE RELATED TO COLLAPSIN. Y. Luo*, M. Renzi and J. A. Raper, Department of Neuroscience, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104.

Collapsin is a recently identified protein (Luo et al. 1993, Cell 25 217-227) that is believed to serve as a repulsive guidance cue during growth cone navigation since it induces the collapse of specific growth cones in vitro, is homologous to the axon guidance molecule fasciclin IV (semaphorin 1), and is expressed in discrete regions of the developing brain. Partially purified brain-derived collapsin induces the collapse of both DRG and retinal growth cones, yet recombinant collapsin induces DRG growth cone collapse without affecting retinal growth cones. We therefore hypothesized that there is a family of collapsin-related molecules in brain that exhibit distinct neuronal target specificities. To identify possible collapsin relatives, we screened a λgt10 chick brain cDNA library with collapsin probe under low stringency conditions. One cDNA clone was identified that hybridizes with the collapsin probe at low stringency (45°C) but not at high stringency (65°C). The 1.6 kb cDNA insert of this clone encodes a portion of a novel protein that is homologous to a part of the fasciclin IV-like and the Ig-like domain in collapsin. In the fasciclin IV-like domain over 70% of the predicted amino acid sequence is identical to that of the corresponding domain in collapsin. We are now in the process of obtaining the full coding sequence for this collapsin-related protein. We then hope to express this recombinant version and test it for collapsing activity on a variety of growth cones in vitro.


CC1 and CC2 are neural crest-derived subunits of vomeronasal (VNO) neurons. Axons from CC1* neurons terminate in the rostral accessory olfactory bulb (AOB), whereas axons from CC6* neurons terminate predominantly in caudal glomeruli of the AOB. In addition, subsets of CC1* and the main vomeronasal nerve and grow caudally toward the forebrain. Many LHRR neurons utilize these CC1* axon branches as guides to migrate along the medial surface of the developing olfactory bulb (OB). Occasionally, CC1* axons also grow caudally toward the forebrain, but turn at the OB/forebrain border and grow back into the rostral AOB. These data suggest that molecules associated with CC1* axons and the terminal arbors of chemically distinct VNO axons. Whole mount immunohistochemical studies indicate that both active and repulsive activities may operate in the developing AOB. Most CC1* axons appear to stop abruptly at the border of the caudal AOB. However, a small number of CC1* axons enter the caudal AOB but alter their direction by turning sharply and entering the rostral AOB. Tissue culture studies are underway to examine factors that influence branching. E18 VNO neurons are grown on coverslips coated with alternating stripes of protein homogenates from neonatal rostral and caudal AOBs. Preliminary results indicate that stripes of axons turn and grow on rostral AOB homogenates but appear to be unaffected by caudal AOB homogenates. Supported by DC00933, NS24386 and HD05515.

533.6 A NOVEL Car++-BINDING PROTEIN IS EXpressed BY a SUBSET OF PERIPHERAL NEURONS WHICH SELECTIVELY FASCICULATE IN A SINGLE AXON TRACT. By LEECH CNS, K.K. Briggs, K.M. Johannsen, & J. Johansen*. Dep. of Zoology & Genetics, Iowa State University, Ames, IA, 50011.

The laf-36 antigen is expressed in a subset of peripheral neurons whose axons reorient to a single axon fascicle in the developing leech CNS. By screening an expression vector library with the antibody we obtained a cDNA clone which encodes a protein possessing two EF-hand Car++-binding domains. The Car++-binding domains are likely to be functional since fusion protein made from the clone and expressed in vivo binds Car++ after SDS-PAGE and transfer to nitrocellulose plates. The Car++-binding ability of the clone co-fractionated with the laf-36 antigen was verified by in situ hybridizations with the clone to embryos, the labeling of which exactly matches the lan 3-6 antibody staining pattern. Northern analysis suggests that translation of the lan 3-6 message would result in a protein with a molecular weight of about 18 kD. Immunofluorescent purification of the lan 3-6 antibody followed by SDS-PAGE and analysis of the gels by silver staining yields a protein band of this predicted size. However, a prominent protein band of approximately 200 kD is being co-purified with the lan 3-6 antigen by this procedure. Broad protein bands of this nature are frequently characteristic of glycoproteins. Thus these results suggest that the lan 3-6 antigen is a small Car++-binding protein which may be associated with and regulating a larger 200 kD glycosylated protein. The very restricted expression of the lan 3-6 antigen to a subset of peripheral neurons which specifically fasciculate together into a single tract during development raises the possibility that this putative protein complex may play a role in this process. Supported by NIH grant NS 28657 and NSF grant DIR 9113909.

533.8 THE PATTERN OF COLLAPSIN mRNA EXPRESSION IN THE DEVELOPING CHICK CNS. S. Chang, Y. Luo, and J.A. Raper*, Department of Neuroscience, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104.

Collapsin is a recently identified protein that induces the collapse and paralysis of specific growth cones in vitro (Luo et al. 1993, Cell 25 217-227). Here we examine the distribution of collapsin mRNA in the developing chick brain. Wholemount in situ hybridizations were performed using a full length digoxigenin derivatized collapsin probe that was later reacted with an alkaline phosphatase conjugated anti-digoxigenin antibody. In the chick brain at E2 (HH stage 1), collapsin message was localized to rhombomeres 3 and 5 of the hindbrain. By E3, the rhombomere expression declines and collapsin message is strongly expressed in specific regions of the diencephalon. It is localized dorsally in an annulus around the developing pineal and ventrally as two strips along either side of the stalk of the pituitary. On E6, collapsin message is also found in a stripe on the lateral wall of the forebrain in a region that may correspond to the archaestriatum. A pair of rostrally-oriented stripes on either side of the midline of the hindbrain also express collapsin message. The expressing cells in these stripes are near the ventricular surface. These two pairs of collapsin expressing stripes extend caudally into the ventral portion of the spinal cord. This highly specific and localized pattern of expression at developmental times when extensive axon outgrowth is occurring in the developing CNS is consistent with collapsin playing a role in growth cone guidance.


Potent contact-dependent axon growth inhibitory activity is present in CNS myelin. To identify inhibitory proteins present in myelin we used non-denaturing chromatography and tested the fractions for growth inhibition in a bioassy. NG108-15 cells extend neurites in response to cAMP. Purified bovine CNS myelin, used as a tissue culture substrate, permitted cell attachment but inhibited cAMP-induced neurite outgrowth. When myelin was extracted with octylglucoside and the extract chromatographed on a DEAE anion exchange column, several peaks of inhibitory activity were eluted with a salt gradient. Analysis of the fractionated proteins revealed that myelin-associated glycoprotein (MAG) co-chromatographed with the bioassay active fractions. To determine if MAG contributes to growth inhibition, MAG was removed from these fractions by immunodepletion. Removal of MAG reduced neurite growth inhibitory activity. Direct evidence for the inhibitory effect of MAG on neurite outgrowth was obtained by taking the extracellular domain of recombinant MAG produced in cultured insect cells. This recombinant MAG was a potent inhibitor of neurite outgrowth. These results, which establish an inhibitory action of MAG through an extracellular domain, suggest that MAG may be critically important after nerve injury in the CNS where myelin debris is not removed very quickly after injury. Supported by the Canadian Centre of Excellence, Neuroscience Network.
MECHANISMS

533.11 NEURITE GROWTH INHIBITORY ACTIVITY IN THE MAMMALIAN PNS AND CNS AND ITS MODULATION BY LAMININ. S. Davis, J. McKerracher, D. Jackson, V. Kottis, and P. Braun. Centre for Research in Neurosciences, Montreal General Hospital Research Institute, and Dep. of Biochemistry, McGill University, Montreal, Canada.

In addition to the axon growth inhibitor in CNS myelin identified by Caroni and Schwab (J. Cell Biol.106:1281), we have identified another inhibitor in the peripheral nervous system (PNS). We now present evidence that peripheral nerve (PN) myelin also possesses neurite growth inhibitory activity. Bovine PN myelin was prepared by homogenization with either a Polytron (PN-p), or frozen in liquid N2, pulverized and homogenized with a Dounce homogenizer (PN-d). These two preparations were then subjected to sucrose density gradient centrifugation to purify myelin. PN myelin was plated on to polylysine-coated 96 well plates (9ug/well), and neurite growth assessed with CAMP linked NG108-15 cells. On PN-p myelin 81% of the cells extended neurites, as compared to 1.5% on PN-d myelin. Western blots of the 2 myelin preparations with anti-laminin antibody indicate substantial amounts of laminin in the myelin preparation that was permissive for neurite growth (PN-p). Preincubation of PN-p myelin with anti-laminin antibody reduced neurite growth. In a similar neurite growth assay, the inhibitory activity in bovine CNS myelin (4%) was overridden (80%) by coating the wells with a mixture of myelin (8ug) and laminin (1.5ug). These results suggest that neurite growth inhibitory activity is present in PN and CNS myelin, and can be modulated by laminin.


Molecular gradients of environmental cues have been proposed to be important directional signals for the proximal growth of pioneer axones in embryonic insect legs. We have produced a monoclonal antibody (Mab) that binds to an extracellular matrix protein that is non-uniformly distributed along the proximal-distal axis of embryonic cockroach leg at the time of pioneer axon growth. This mab was named PROD-2 because it labels the proximal parts of the leg more intensely than the distal end. The spatial and temporal distributions of the PROD-2 antigen were used to generate an environmental guidance cue for T11 pioneer axon growth. To demonstrate a role for the antigen in axon guidance, PROD-2 Mab is added to the medium in which whole embryos are cultured for 48 hours. Under control conditions, the T11 axons grow and follow their normal path. Treatment with PROD-2 Mab has no effect on the rate of elongation of the T11 axon, but alters the direction in which they grow. The perturbation was found to be dose dependent; with increasing concentration of the Mab there was an increase in the total number of pathfinding mistakes as well as an increase in the severity of the mistakes. These results indicate that the PROD-2 antigen is a guidance cue responsible for the stereotypical projection of the T11 pioneer axons on their normal substrates.

533.15 AXONIN-1 PLAYS A ROLE IN GUIDANCE OF COMMISURAL NEURONS IN THE CHICK SPINAL CORD. E.T. Stoeckl* and L.T. Landmesser. Dept. of Neurosciences, Case Western Reserve University, Cleveland, OH 44106

In chick embryo the cell adhesion molecule axonin-1 has been shown to exist in both a secreted and a GPI-linked form. Its role in growth cone guidance and pathfinding of commissural neurons, which express it during initial axonogenesis, was tested by in vivo injections of axonin-1 into the spinal cord central canal in early embryos (Stages 18-24). Dorsally located commissural neurons normally extend axons toward the floor plate in response to a diffusible factor. Upon reaching the floor plate they make a sharp turn to cross the midline, then turn rostrally along the border of the contralateral floor plate. In axonin-1 injected embryos, a substantial proportion of commissural axons did not cross the midline, but turned rostrally on the ipsilateral side; in addition they grew in a morechaotic pattern (antero-posterior). When Neurocan-CAM has been shown to be a receptor for axonin-1 on DRG neurons, we tested whether injection of Fab fragments of anti-NcCAM would result in similar pathfinding errors. Although commissural axons grew in a more defasciculated pattern following anti-NcCAM injection, pathfinding errors did not occur. This suggests the existence of at least one other receptor for axonin-1 in the floor plate; saturation of this "floor plate" receptor by injected axonin-1 could lead to the observed misguidance of commissural neurons. The nature of this receptor is currently being investigated.

533.16 SUBSTITUTES PATTERNED WITH A DOMAIN OF THE B2 CHAIN OF LAMININ GUIDE AXONS OF EMBRYONIC HIPPOCAMPAL NEURONS IN VITRO. M. Matsuura*, B. S. Potember*, F. E. Weight* and P. Ledis. 1. Applied Physics Lab, The Johns Hopkins University, Laurel, MD 20723. 2. Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Substrates patterned with synthetic peptides derived from the B1 and B2 chains of laminin were used to guide axons of hippocampal neurons in vitro. These substrates were formed by attaching the peptides to chemically patterned surfaces fabricated via deep UV lithographic procedures. Neurons grown on the patterned substrates (parallel lines of 5 μm width) with or without the B2 peptide, or without substrate (control), had neurite outgrowth domains. The 60% of the neurons on patterned substrates had randomly spreading neurites. Substrates patterned with peptide P20, derived from a neurotrophic factor domain of the B2 chain of laminin, had a morphology similar to that of the control, but was not significantly different from the B2 peptide. The patterned substrates had a dose-dependent effect on neurite outgrowth. Higher doses of peptide P20 promoted directed neurite outgrowth and morphological maturation of neurons. Neurons on patterned substrates had an average 4.4 μm length, compared to 5 μm length of the control. These results demonstrate that patterned substrates can be used to influence neurite outgrowth and axon differentiation. The future study will provide direct evidence that the integrative cell surface domain of a peptide, which is derived from the B2 chain of laminin as an axonal growth factor, is required for functional growth of hippocampal neurites in vitro.


533.17 AN INSECT PIONEER NEURON USES MESODERMAL CELLS AS A SUBSTRATE FOR NORMAL PATHFINDING. R. J. Bajant* and J. G. Denburg. Dept. of Biological Sciences, University of Iowa, Iowa City, Iowa 52242.

Previous studies of axon growth of insect pioneer neurons by Bentley’s lab has shown that mesodermal cells are not required for elongation and pathfinding. We report that the extension of axonal neurites in a stereotypical axon projection by the Fe2 pioneer neurons in the limbs of cockroach embryos requires mesodermal cells. Using immunocytochemical and confocal microscopy techniques, we have observed that the Fe2 pioneer neurons use the internal core of mesodermal cells as a substrate for growth. Surgical removal of the leg mesoderm and subsequent culturing of the embryos resulted in axonal growth on the epidermal epithelium but with an altered trajectory. Similar changes in path and substrate choice are observed in axons growing in embryos cultured in vitro in the presence of the enzyme phosphatidyl inositol phospholipase-C or certain glycolipids similar to those in epidermal cells. These results indicate that in vivo the Fe2 axons prefer to grow on the surface of certain mesodermal cells. However, after surgical manipulation or addition of chemical perturbants they will grow on ectodermal epithelial cells but with an altered path. There appears to be a direct effect of epidermal cells on neurite outgrowth mechanisms of pioneer axon growth in the legs of insect embryos. Supported by NIH grants NS 14293.
533.17 Substance P Modulates Chemotactic Influences of Floor Plate on Developing Neurites in the Central Nervous System. C. De Felice, R.D. Pincock* and S. T. Hunt,* (Spon. BRA) MRC Laboratory of Molecular Biology, Division of Neurobiology, Hills Road, Cambridge, CB2 2QH, Tisch–Davis Neuroscience Research Unit, Robinson Way, Cambridge, UK CB2 2QB.

The floor plate, a neuro-epithelial structure found at the ventral midline of the developing spinal nervous system, has been shown to function in dorsal-ventral patterning in the spinal cord and at later embryonic stages, in guidance of commissural axons by release of chemotactants. We have found that embryonic rat floor plate cells express the neurokinin 1 receptor and that there is a transiently appearing subpopulation of commissural axons containing Substance P, the neuropeptide ligand for this receptor. Receptor coupling to an intracellular effector system in dissociated floor plate cells was established by monitoring the intracellular calcium levels of dissociated floor plate cells using the Ca2+ indicator fura-2. Both application of 10 to 100 nM substance P or the selective NK1 receptor agonist [Sar^5, Met(O2)^8] substance P (12) produced a rapid and reversible increase in internal calcium levels which was concentration dependent. Prolonged application of sub-threshold concentrations (0.1-5 nM) of substance P applied for up to 10 min produced rhythmic oscillations in internal calcium levels. The NK2 and NK3 receptor agonists [B-Ala^9]‐prokineticin A (4-10) and sendikin (100 nM) were inactive. 50 nM concentrations of the rat selective NK1 receptor antagonist CP967580 blocked the response to substance P. Finally, Substance P analogues increased the amount of axon outgrowth from dorsal horn explants when co-cultured with floor plate in collagen gels. These results suggest that SP released from pioneering neuronal pathways can subdue the extracellular environment and influence the growth and differentiation of developing neuronal systems by regulating the release of chemotactants from floor plate cells.


Floor plate cells at the ventral midline of the spinal cord of early neurulae (stage 11) promote outgrowth of spinal commissural axons and reorient these axons in vitro. We recently identified two proteins, netrin-1 (which is highly expressed in floor plate) and netrin-2, that are expressed at lower levels in the floor plate and that promote spinal axon outgrowth-promoting and reorienting activities of the floor plate. Here we report the characterization of a chemically distinct activity that potentiates the effects of the netrins. This activity, termed NSA (for netrin synergizing activity), was found in high salt extracts of embryonic brain membranes that had been depleted of netrins by passage over a heparin column, at high salt. On young E11.5 rat embryo spinal cord slices, addition of NSA increased the potency of the netrins in promoting commissural axon outgrowth by one order of magnitude. Surprisingly, commissural axons in older (E13) spinal cord were more sensitive to the netrins, and, conversely, NSA had relatively little effect at this age. The reasons for the differences in responsiveness of commissural axons of different ages is not known but could be explained if E13 dorsal spinal cord produces NSA.

To determine whether the NSA potentiating observed with E11 dorsal spinal cord is a true synergy, we identified a concentration of the netrins that, alone, evoked relatively little outgrowth from E11 explants, just as the standard concentration of NSA evoked little or no outgrowth. At E11 this concentration of NSA is only about half these defined concentrations, robust outgrowth was observed which cannot be accounted for by a simple additivity of their effects. Initial experiments revealed that NSA is in prototype sensitive and has a molecular weight in excess of 100 kDa. Purification and identification of NSA will make it possible to determine the mechanism of the synergy and to determine whether NSA modulates netrin function and contributes to commissural axon guidance in vivo.

534.1 NEUROTROPHINS POTENTIATE NEURONAL EXCITABILITY BY INHIBITING GABAERGIC SYNAPTIC TRANSMISSION IN CORTICAL NEURONS. Han G. Kim*, Ti Wang and Bai Lu, Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey.

Neurotrophins (including NGF, BDNF, NT-3, NT-4/5) have traditionally been regarded as slowly acting signals essential for neuronal survival and differentiation. A recent study showed that BDNF and NT-3 can rapidly potentiate synaptic activity in developing neuromuscular synapses. However, little is known about the role of neurotrophins in modulating excitability of the developing central nervous system. Here we report that NT-3 and NGF rapidly increased the frequency of spontaneous action potentials, and synchronized synaptic activities in developing cortical synapses. In addition, the inhibitory synapses, which are mediated by GABA receptors, were reduced by NT-3. Thus, the excitatory effects of neurotrophins on spontaneous action potentials were antagonized by the induction of GABAergic transmission. Our finding, together with previous report of the rapid regulation of CNS neurotrophin expression by neuronal activity, suggest a new mechanism for modulation of synaptic transmission and activity-dependent synaptic plasticity.

534.2 NGF, a Synaptically Released Protein with Homology to Acute Phase Proteins of the Immune System. A.K. Schlingman, J.A. Holmes, H. Vogel, and M.S. Perie*, Division of Neuroscience, Dept. of Otorhinolaryngology, Dept. of Pathology, Baylor College of Medicine; Houston, Texas 77030.

We have identified by affinity chromatography, a binding protein for the snake venom toxin, Taipoxin. The sequence of this 47 kD protein is unique, is suggestive of a secreted protein and has homology to the acute phase proteins, serum amyloid P protein and C-reactive protein, of the pentraxin family. Northern analysis revealed a brain specific transcript. In vitro hybridization shows this high NGP message levels in neurons of the cerebellum, CA3 and dentate gyrus of the hippocampus, layer 6 of the cortex with no message in gila. Antibody to this protein stains synapses in the cerebellar Purkinje cell somata and Bergmann glomeruli and a subset of gila. Reflecting this, we have named this protein, Neuronal-Glial Pentraxin or NGP. Because NGP appears to be released synthetically and has homology to immune proteins potentially involved in uptake of lipids, toxic or other antigenic material, we suggest that NGP may be involved in the uptake of synaptic molecules during synaptic remodeling.
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Little is known about the regulation of expression of the growing number of proteins identified as possible regulatory factor could be the level of neuronal activity, which in sensory systems is dependent on the level of afferent stimulation. We examined the effects of unilateral suppression of odor-mediated stimulation to the olfactory bulb (OB) on 3 synaptic vesicle proteins (SVPs): synaptophysin, SV2, and rab3a, a small GTP-binding protein of the rab family.

The right nostrils of six newborn rats were cauterized under anesthesia. The animals were sacrificed 6 weeks later, their brains removed and the left and right OBs immunostained with specific antibodies against 3 SVPs: Tyrosine hydroxylase (TH) immunohistochemistry served as a control for the efficiency of the sensory deprivation. Sections were digested and deionized by analysis performed in the glomerular (GLOM), the internal plexiform (PLEX) and the granular (GRAN) layers. The level of staining in the striatum was used as an internal standard to normalize between animals.

TH staining was reduced in the GLOM (-30%, p<0.001) but not in the other layers. Also, the signals of synaptophysin and SV2 were reduced or unchanged. In contrast, staining for synaptophysin and SV2 was similar in all layers in the left and right OBs. These results demonstrate that the level of expression of rab3a, but not of synaptophysin or SV2, are influenced by the afferent input to the OB. One possible interpretation is that rab3a would act as a regulatory element of TSP trafficking, while synaptophysin and SV2 could be regulated more as "constitutive" components.

P54.4 LONG TERM MAINTENANCE OF NERVE TERMINAL FUNCTION IN THE ABSENCE OF MUSCLE FIBERS IN THE FROG. A. Dunaevsky and E. A. Connor* Neuroscience and Behavior Program, Univ. of Massachusetts, Amherst, MA 01001.

Although some of the signals involved in forming and maintaining the synaptic specializations of a target cell are now identified, little is known about the processes that underlie and maintain the presynaptic nerve terminal. Here the role of the muscle fiber in the maintenance of nerve terminal function has been investigated. We demonstrate that motor terminals specifically deprived of targets maintain the ability to release and recycle synaptic vesicles in response to stimulation. We assayed nerve terminal activity in preparations of innervated muscle basal lamina sheets using a fluorescent dye FM1-43 that stains nerve terminals in an activity-dependent fashion. Intervened basal lamina sheets were prepared by exciting cutaneous dermonecrotic mouse fibers with stimulated nerve fibers. X-ray irradiation prevented regeneration of the muscle fibers. One to five months after muscle fiber excision, the function of target deprived nerve terminals was tested by exposure to FM1-43 dye in high K* Ringer's solution. Synaptic sites were identified with rhodamine-labeled peanut agglutinin which stains terminal Schwann cell and synaptic basal laminae. Like intact preparations, nerve terminals deprived of target for 1-5 months incorporated FM1-43 when stimulated. Further, the same terminals were destined by either nerve stimulation or depolarization with high K* Ringer. The ability of these nerve terminals to release FM1-43 depended on the target deprived nerve terminals.


Ecto-protein kinases provide the powerful regulatory machinery of protein phosphorylation to the extracellular environment of the nervous system, where delicate interactions among cells, and between surface proteins and components of the extracellular matrix, play significant roles in neurogenesis and synaptogenesis. The present study identified proteins whose surface phosphorylation corresponds to these developmental events. Cultured, embryonic chick-brain neurons possess proteins of MW 12k and 13k whose surface phosphorylation was maximal during the onset of neurogenesis. Surface phosphorylation of this low MW protein duplex was delayed only in the development of pyramidal neurons cultured from embryonic mouse hippocampus, and this activity decreased after neurogenesis was completed. Upon maturation and during synaptogenesis, the phosphorylation of another protein duplex (MW of 4DK and 50kD) increased. As anticipated from the results listed above, we found that a hybrid, pyramidal-neurons cell line (HN30) derived from embryonic cells has high phosphorylation of the low MW protein duplex associated with neurogenesis, and a more mature line (HN33) displayed the high surface phosphorylation of the 4DK-50kD protein duplex, associated with synaptogenesis.

The functions of these surface protein phosphorylation systems can now be studied in homogenous populations of cloned neural cells.

P54.6 PHOSPHORYLATION OF EXTRACELLULAR DOMAINS OF MICROTUBULE-ASSOCIATED PROTEIN (MAP) 1B MAY BE INVOLVED IN SYNAPSE FORMATION BETWEEN CORTICAL NEURONS. K. Muramoto*, M. Kakehara, K. Kobayashi, H. Taniguchi and Y. Kuruda, Department of Molecular & Cellular Neurobiology, Tokyo Metropolitan Institute for Neuroscience, Fuchu-shi, Tokyo 183, Japan. and Division of Biomedical Polymer Science, Institute for Comprehensive Medical Sciences, Fuji Health University, Toyoake-shi, Aichi 470-17, Japan.

Synapse formation between cultured rat cortical neurons is inhibited by the continuous application of K-252b, an ecto-protein kinase inhibitor, which can not permeate the cell membrane (Muramoto, et al., Proc. jap. Acad., 64, 319, 1988; Kuruda, et al., Neurosci. Lett., 135, 255, 1992). In order to identify membrane proteins which may be involved in synapse formation, [32P]P/ATP was applied to the cultured cells for brief periods to phosphorylate their extracellular domains. The phosphorylated proteins were separated by SDS polyacrylamide gel electrophoresis and detected by autoradiography. Some of these bands were immediately phosphorylated and this phosphorylation was suppressed by the addition of K-252b to the medium. We examined the partial amino acid sequence of these substrates. Phosphorylated bands were cut from the gel and digested with lysyl endopeptidase. Peptide fragments were separated by capillary HPLC and analyzed by mass spectrometry. The band with the highest molecular weight, whose phosphorylation was strongly inhibited by K-252b, was identified as microtubule-associated protein (MAP) 1b. These results suggest the possibility that the phosphorylation of extracellular domains of MAP1b in involved in synaptic formation between cortical neurons.


We have used immunocytochemical techniques (De Vente et al., 1997, Neurosciences 23:361) to monitor cGMP changes in identified neurons in Locusta migratoria in response to agents such as sodium nitroprusside (SNP) that generate nitric oxide (NO). Although relatively few neurons respond to SNP during the embryonic and larval stages, a number of neurons show a window of NO sensitivity during embryogenesis. Observations on identified neurons such as RP2, ACC and LPD cell show that NO sensitivity development begins after the completion of axon navigation, is strong throughout axonal branching, and ends after synaptogenesis is apparently completed. During this embryonic window, the main site of cGMP accumulation is the nucleus, possibly providing a direct link between cell-cell signaling and transcription. Using nonradioactive tracing to detect possible sites of NO synthesis, we found diaphorase staining first associated with nerve fibers at 40-45% of development. This was increased in intensity in the neuropil through the remainder of development. These observations suggest that the maturation phase of neurons is a period uniquely characterized by the NO/cGMP signaling system and of nuclear targets for cGMP. NO-synthesizer inhibitor experiments are being used to define the exact role of this system in neuronal maturation.

P54.8 BLOCKADE OF SPONTANEOUS NEURAL ACTIVITY IMPAIRS THE DEVELOPMENT OF THE DENDRITIC SPINES IN CULTURE. Teng Wu*, Mark J. Maguire, Ford F. Ember, Institute for Developmental Neuroscience, John F. Kennedy Center, Vanderbilt University, Nashville, TN 37203.

Blocking patterned neural activity has been shown to prevent the normal development of dendritic spines in vivo. We have investigated whether spontaneous neural activity is necessary for induction of spine formation in vitro. Neurons from rat somatic sensory cortex were enzymatically dissociated on postnatal day 1 and plated on a glial monolayer. Pyramidal neurons were cultured in a medium containing TTX (1 uM), B27, EGF (5 ng/ml), and BSA (1 mg/ml) for 2 weeks. In the presence of TTX, spine density on these neurons was significantly decreased compared to neurons cultured in the same medium without TTX. However, after 4 weeks of culture, spine density on neurons cultured with TTX were not significantly different from neurons cultured without TTX. These results suggest that the formation of dendritic spines is critically dependent upon spontaneous neural activity.

(Supported by NS-13031)
Formation and Specificity of Synapses IV

S53.1
Embryonic Epinephrine Synthesis: Association with Cardiogenesis. S. H. Ebert, M. Fujimura, J. M. Baden, B. Sidel and D. P. Luskin. Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305.

Phenylethanolamine N-methyltransferase (PNMT) is the final enzyme in the pathway for epinephrine synthesis; hence, PNMT serves as a marker for the cells which produce this neurotransmitter/neutrotransmitter. While much is known about the developmental expression of PNMT in the adrenal gland and the brain, very little is known about its developmental expression elsewhere. We demonstrate that PNMT mRNA can be detected in embryos as early as gestational day 9.5. By gestational day 11, PNMT mRNA and active enzyme can be specifically localized to the heart. Immunocytochemical analysis further reveals that PNMT protein is predominately contained within the embryonic cardiac cells. Our results therefore suggest that the embryonic heart is the primary site of PNMT expression early in development. The presence of PNMT mRNA, enzyme activity and protein before the appearance of nerve cells around the heart suggests that the embryonic heart may be capable of synthesizing epinephrine during early cardiogenesis. Since cardiac cells are already beating and can respond to epinephrine at this stage of development, our findings indicate a potential role for epinephrine in early cardiac function.

S53.2
Neonatal 6-OHDA Lesions Affect the Development of Striatal D1, D2, D3 Receptors and D1 mRNA. P. A. Frohna*, B. S. Natoli-Reisvogel, and J. N. Jessen. Dept. Psychiatry and Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

The postnatal developmental profile of striatal dopamine reuptake and dopamine receptor expression suggests that the two may be related. Our previous studies have shown that adult rats that received postnatal day 6 (P6) 6-OHDA injections had a significant loss of D1 receptors that peaked at 7 days after the lesion. This peak in D1 mRNA in the dorsolateral striatum (DLS), substriatal (MS) and medial (MD) dopamine (DA) enriched cortex (Crp), regions with the greatest density of D1 receptor-positive fibers. Thus, normal DA innervation during postnatal development may be important for the appropriate control of motor function. In the present study, we determine the temporal relationship between the loss of DA innervation and the development of specific DA receptor expression by the striatum. The profile of D1 receptors and D2 and D3 mRNA in rats with P1-6-OHDA lesions.

S53.3
Neonatal 6-OHDA Lesions Affect the Development of striatal D1, D2, D3 receptors and D1 mRNA.

S53.4
PERINATAL INSULTS TO DEVELOPING DOPAMINE SYSTEMS IN RAT. K-C. Langley* and C.C. Quinn. Program in Neuroscience, Florida State University, Tallahassee, FL 32306.

Perturbation of developing dopamine systems in neonatal rat brain may lead to cytoarchitectural restructurin that persists into adulthood. Such disruptions allow for an opportunity for the animal to assess the effects of environment and to provide an opportunity for the animal to assess the impact of environmental cues on the developing brain. The results of this study suggest that the development of striatal D1 and D2 receptors in neonates is differentially regulated, and that D1 receptors require normal DA innervation for proper expression. This work supported by MH 48813.
The effects of dopamine receptor agonists on the expression of dynorphin (DYN) and Fos or Fos related antigens (FRA) were studied in primary striatal cultures from 7-day old rat pups. Levels of DYN, c-fos, and FRA were increased in cultures exposed to dopamine receptor agonists, at 10 μM (one day administration) resulted in an increase in the number of DYN-positive cells in the striatal cell culture and increased the intensity of DYN mRNA in cell nuclei. Compared to control or quinpirole, a specific D2 dopamine receptor agonist, at 10 μM (one day administration) resulted in an increase in the number of DYN-positive cells in the striatal cell culture and increased the intensity of DYN mRNA in cell nuclei. Compared to control or quinpirole, a specific D2 dopamine receptor agonist, at 10 μM (one day administration) resulted in an increase in the number of DYN-positive cells in the striatal cell culture and increased the intensity of DYN mRNA in cell nuclei. Compared to control or quinpirole, a specific D2 dopamine receptor agonist, at 10 μM (one day administration) resulted in an increase in the number of DYN-positive cells in the striatal cell culture and increased the intensity of DYN mRNA in cell nuclei. Compared to control or quinpirole, a specific D2 dopamine receptor agonist, at 10 μM (one day administration) resulted in an increase in the number of DYN-positive cells in the striatal cell culture and increased the intensity of DYN mRNA in cell nuclei. Compared to control or quinpirole, a specific D2 dopamine receptor agonist, at 10 μM (one day administration) resulted in an increase in the number of DYN-positive cells in the striatal cell culture and increased the intensity of DYN mRNA in cell nuclei.
BRAIN AND ENDOThERmAL NICRiC OXIDE SYNThESE EXPRESSION IS REGIONALLY AND TEMPORALLY REGULATED IN FETAL SHEEP BRAIN. F.J. NorthIton, R.C. Koehler, R.J. Traysman*, and L. J. Martin*. Dept. of Pediatrics, Anesthesiology/Critical Care Medicine, Pathology, and Neuroscience. The Johns Hopkins Medical Institutions, Baltimore, MD 21287.

Using immunohistochemistry, we tested the hypothesis that brain NOS (bNOS) and endothelial NOS (eNOS) expression is regulated developmentally in rat, 70, 93, 110 and 136 days of gestation (term=145d). No nonspecific immunoreactivity (IR) was found if the IR antibody was omitted; no cross reaction between bNOS and eNOS was seen at any gestational age, and neither isomer was localized to glial cells. At 60d, bNOS has a laminar localization in neopil of the lower cortical plate of the telencephalic vesicle which becomes less distinct as the cortical plate matures, disappearing at 136d. A subset of nonpyramidal cortical neurons first shows discrete bNOS IR at 71d. By 136d, two subsets of interneurons with somatolaminar IR for bNOS are dispersed throughout cerebral cortex. Neuronal differences in bNOS IR within cortex occur at 71 and 93d but not at 110 and 136d. IR for bNOS in striatum increases from first appearance at 71d to peak density of bNOS-positive medium sized aspy neurons at 110d. Labeling of cerebellar basket cells peaks at 136d. The vascular bed expresses eNOS throughout gestation. The radial vascular architecture evolves into a complex branching pattern between 60 and 110d. A dense capillary bed with eNOS IR is found within white matter in contrast to the relative absence of bNOS IR in this area. By 136d, eNOS in vascular beds of brainstem, diencephalon, basal ganglia, and cortex is homogeneously distributed, in contrast to the absence of eNOS IR in the ventricular zone. We conclude that bNOS and eNOS are present prior to midgestation in the fetal sheep, and the developmental regulation of NOS expression, particularly bNOS, parallels regional brain maturation.

REGULATION OF EXPRESSION OF NEURODianeAcPH ACTIVITY (NDA) AND NEURODianeAcPH SYNThESE (NOS) IN PRINCIPAL NEURONS OF RAT AND MOUSE SUPERIOR CERvICAL GANGLIUM. MD Johnson*, JA Garcia,* BA Senators, DG Green*, and JD Potter*. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Preganglionic sympathetic axons of adult rats and mice express NADPH-D (NDA) and NOS immunoreactivity in vivo; however, principal neurons of the superior cervical ganglion (SCG) express these markers for NOS lightly or not at all (E. Grekou et al, 1992, Neuroscience 58:255; Du et al 1993, Neurosci. Lett. 158:51; Johnson, et al, unpublished). When postnatal rat and mouse SCG neurons (and some adult SCG neurons) were cultured for 1 week in medium containing 5% rat serum, however, NDA-D was visibly induced in about 80% of such cells; the staining varied from barely detectable to intense (Senatius et al., 1992, Abstract B777). In a subset of neurons, NADPH-D was co-localized with immunostaining for the neuronal isoform of NOS (antibody provided by Dr. Bernd Mayer). In mice homotypic for deletion of the neuronal NOS gene (provided by Dr. Paul Hobart, of Houston, et al, 1993, Cell 72:237), and in which there was no NADPH-D in the preganglionic sympathetic fibers in vivo, clear light NADPH-D was present in principal neurons in adult ganglia, and in cultured postnatal neurons; the staining of the cultured neurons was significantly lower than in neurons from wild-type mice. The presence of numerous ganglionic non-neuronal cells in the cultures reduced the proportion of NADPH-D stained cells. Supported by NS02253 and the Feinstein Fund. We thank Kara Dunn for technical assistance.

EFFECTS OF SERA ON INDUCTION OF NEURODianeAcPH ACTIVITY (NDA) IN CULTURED PRINCIPAL NEURONS OF THE SUPERIOR CERVICAL GANGLION (SCG) OF THE POSTNATAL RAT. J.A. Garcia, M.D. Johnson* and D.D. Potter*. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Induction of NADPH-D was reported in dissociated and cultured SCG neurons of the postnatal Long Evans rat by Senatius et al., 1992. Characterization of the time course and level of induced NADPH-D in cultured SCG using growth medium with 5% normal rat serum (NRS, Sigma) demonstrated NADPH-D in less than 1% of SCG neurons at time of plating and in about 80% of neurons after 1 week in culture. The inductive effects of 5% NRS have now been compared to growth medium supplemented with 5% dialyzed fetal calf serum (dFCS, 10,000 MW cut-off). SCG neurons cultured in 5% dFCS also displayed a positive inductive effect on NADPH-D, but the percentage of neurons expressing NADPH-D was significantly lower (<1% at time of plating and about 30-40% after 1 week in culture). Culture in a serum-free medium resulted in 15-20% of neurons expressing NADPH-D after 1 week, and culture in growth medium supplemented with 5% heat inactivated serum resulted in 20-25% of neurons expressing NADPH-D after 1 week.

To characterize further the time course and nature of these positive inductive effects, a serum reversal experiment was performed. Neurons cultured in 5% NRS were switched to 5% dFCS and vice versa. Serum reversal on culture Day 19 had no subsequent effect on the percentage of NADPH-D positive neurons. Serum reversal on culture Day 6-7 had the following effects: neurons cultured first in 5% NRS and later switched to 5% dFCS maintained an 80% level of NADPH-D (no change); however, neurons cultured first in 5% dFCS and later switched to 5% NRS went from a 30-40% NADPH-D level to an 80-85% level of NADPH-D. These data suggest that the factor(s) in serum that promotes induction of NADPH-D is heat labile and is effective under these conditions in only a limited period in culture. Supported by PO1 NS 02253 and the Feinstein Fund.

MEASUREMENT OF NEUROTENSIN (NT), NT-11-1 AND NT-11-8 IN THE DEVELOPING RAT BRAIN. H. Moss, G. W. Benoit and C. A. Mandsager (SPON: Brain Research Association) Dept. Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, UK.

Neurotensin (NT) appears to be a neurotransmitter/neuromodulator in the adult rat brain, whilst in the developing rat brain, an early transient appearance of NT precursor mRNA in some brain regions (Sato et al. (1990) Dev. Brain Res. 54:249-255), suggests a developmental role for NT, perhaps in the establishment of neuronal organisation.

In the present study NT-LI has been quantified in the diencephalon, telencephalon and ventral mesencephalon of Wistar rat brains aged from embryonic day 15 (E15), adult (3-4 months) cultured for 1 week to postnatal day 2 (P2), using a sensitive radioimmunoassay (RIA) technique (0.9 fmol/tube).

NT-like immunoreactivity (NT-LI) appeared during the prenatal period in all three regions studied and exhibited two general ontogenic patterns. In the diencephalon and telencephalon NT-LI levels peaked at birth and E20, respectively, then declined, whilst in the ventral mesencephalon, where levels were approximately 25% of those in the diencephalon and telencephalon, NT-LI after an initial increase reached a plateau around E18, which was maintained through to P2.

The polyclonal NT antibody was N-termally directed and then recognised and measured the NT metabolites, NT-11 and NT-11, in addition to NT itself. Therefore, a High Pressure Liquid Chromatography (HPLC) method, utilising a method gradient from 30% to 60% over 30 minutes, was developed to separate these three peptides prior to measurement by a RIA of increased sensitivity (0.3 fmol/tube). The latter two techniques are thus presently being used to quantify the proportions of NT, NT-11 and NT-11 in foetal rat brain regions.
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CATECHOLAMINERGIC MEDIATION OF COCAINE-INDUCED INCREASE IN REGIONAL LEVELS OF bFGF mRNA. D. Mucio, J. I. Nocetti, and K. Gale. Departments of Pharmacology and Cell Biology, Georgetown Univ. Medical Center, Wash., DC. 19907.

The behavioral stimulant response produced by cocaine is known to show a long-lasting increase with repeated administrations. Such long term plasticity is believed to be the result of neurotrophic factors. We have previously shown that the mRNA encoding basic FGF (bFGF) is increased in selected brain areas after a single, prolonged cocaine exposure. In this study, we examined the neurotransmitter receptors that mediate this effect. Rats (450g) received 40 mg of cocaine over 8 h, after which they were sacrificed and bFGF mRNA was measured. Cocaine induced a 3.5-fold increase in bFGF mRNA in striatum, substantia nigra and frontal cortex. Pretreatment with haloperidol (3.3mg/kg) or chlorpromazine (20mg/kg) prevented the cocaine-induced increase in bFGF mRNA. However, pretreatment with either pinemide (2mg/kg), prazosin (1mg/kg), or a low dose of chlorpromazine (2mg/kg) did not attenuate the cocaine-induced effect on bFGF. Pretreatment with propranolol (20mg/kg) was likewise ineffective. It therefore appears that concurrent blockade of dopamine receptors and noradrenergic alpha receptors is necessary for preventing the induction of bFGF mRNA expression in response to cocaine. This suggests that cocaine may influence neurotrophic factor expression via either dopaminergic or alpha adrenergic mediated processes. Studies in progress with selective alpha blockers and selective dopaminergic receptor antagonists will more precisely identify the relative contribution of these receptors to the changes in bFGF mRNA levels that follow cocaine exposure.


There is evidence that thyroid hormone (TH) is required for the differentiation and maintenance of septal cholinergic neurons. However, well-documented TH-induced changes in TH deprivation of basal forebrain cholinergic neurons. In previous studies, chronic treatment with TH (0.5 mg/kg) induced an increase in TH-binding sites, but not the cortex, of aged rats (Giordano et al., 1992). Our interest was to determine whether the effects of TH on cholinergic cells are mediated through induced NGF. RNase protection analyses of NGF mRNA after acute systemic administration of various doses of either T3 or T4 revealed induction of NGF in the hippocampus and cortex, but not in the septal region of adult Sprague Dawley rats. Peak increases (approximately 1.5-fold) occurred at 3 h and 3.5pg/kg with T3 and T4, respectively. The effect of each hormone on NGF synthesis was shown to be stereospecific. TH induced BDNF as well as NGF in the cortex, and the induction of both neurotrophins was sustained for at least 30 h. Evaluation of TH in the inmnia fornix lesion model of basal forebrain cholinergic neuronal degeneration will also be discussed.

REGULATION AND POLARITY OF NEUROTROPHIN SECRETION. Laura J. Goodman and Franz Hufn. Department of Neuroscience, University of California, San Francisco, CA 94120.

The neurotrophins, BDNF and NT4/5 are factors that regulate the growth and survival of selected populations of peripheral and central nervous system cells. In this study the regulation and polarity of neurotrophic factor secretion was investigated. Analysis of expression of either BDNF or NT4/5 following expression in the pheochromocytoma-derived cell line, PC12, resulted in the constitutive secretion of immunoreactive proteins migrating at a molecular weight identical to mature BDNF and NT4/5. In the presence of KCl (100mM), secretion of NT4/5 from PC12-transfected cells increased 10-fold as determined by radio/binding/imunoprecipitation and western-blot analysis. This indicates that the protein is primarily expressed by a regulated pathway of secretion. As an initial approach to understanding the polarity of neurotrophic factor secretion, the release of BDNF and NT4/5 was investigated in the polarized epithelial cell line, MDCK. Previous studies have determined that many of the same protein sorting mechanisms exist in MDCK cells as do in polarized hippocampal neurons in vitro. Cells expressing BDNF or NT4/5 were grown on transwell inserts to assess the potential for differential secretion occurring high electrical resistance. Under these conditions, 60-90% of the BDNF secreted from transfected MDCK cells was released from the apical domain, whereas NT4/5 was secreted from both basolateral and apical domains. This suggests that BDNF may be released primarily from the apical domain in polarized hippocampal neurons. We are currently testing our hypothesis in transfected hippocampal neurons grown in vitro.

ALTERNATE STRIATAL NEUROTROPHIC MGF LEVELS IN HYPOTHYROID WEAVER MICE MARIANNE BLUM*, Emilie Garasco, Nancy Gomez, and Shoshana Weidenthal. Fishergen Research Center for Neurobiology, Mount Sinai School of Medicine, New York, NY 10029.

The degeneration of nigrostriatal dopamine neurons that occurs in the weaver mutant mouse can be viewed as failed postnatal development. For example, neuronal death is a consequence of normal transition from a primarily patchy innervation to a more diffuse innervation of the striatum during the first weeks of postnatal life. It is possible that insufficient levels of target-derived neurotrophic support is responsible for the abnormal dopaminergic neuronal development and degeneration. Therefore, we measured the levels of the mRNAs encoding two putative dopaminergic trophic factors using a quantitative nuclelease protection assay. Unexpectedly, we observed that while the levels of BDNF and GDNF mRNA levels are elevated in the weaver mutant striatum relative to controls (p<0.05 and p<0.01, respectively), we did not observe a change in the weaver striatum appears to develop abnormally, and is also body growth is stunted and their eyelid opening is delayed. Since body growth rate and timing of eyelid opening are regulated by thyroid hormone, we hypothesize that weaver mice are hypothyroid. Using radioimmunopurification, we determined that the mutant mice have significantly lower thyroid hormone (T4) levels during the third postnatal week of life (p<0.001). This corresponds to the time when the development of mutant dopaminergic neurons is arrested. We speculate that the reduction in thyroid hormone, which is a potent regulator of brain development, could account for altered trophic factor mRNA levels and ultimately altered dopaminergic neuronal development.

NGF RECEPTOR low affinity IMMUNOSTAINING IN CHOLINERGIC NEURONS DURING ADULT HYPOTHYROIDISM. J. C. Cabib, S. E. Alte, J. L. Landscape, and D. R. Levi-Monzon. Inst. Of Human Physiology, University of Cagliari; SInst. of Neurobiology, CNR, Rome, Italy; Inst. Otolarygol., University of "Milano" and #Monza, Italy.

Adult hypothyroidism is characterized by neurochemical abnormalities involving neurotransmitters, neuropeptides, neuronal proteins and also trophic factors. Several clinical and experimental data suggest that the cholinergic synapses of the basal forebrain are sensitive to the peripheral levels of thyroid hormones. In this paper we investigated the NGF receptor low affinity (LNGFR) immunostaining in the normal and hypothroidic forebrain. LNGFR was labeled with a monoclonal antibody (clone IgG192, generously supplied by E.M. John.). The staining intensity was measured by means of computerized microdensitometry. In colchicine untreated rats, we found a significant increase of LNGFR-like immunostaining in the basal forebrain neurons of hypothryoid rats. The colchicine treatment increases an increase of LNGFR expression in these brain area which is not different in hypo- and hyperthyroid rats. These data confirm an effect of thyroid hormone on neurons during adulthood also in extrahypothalamic areas and they suggest a possible role of thyroid hormone in aging brain quality and cognitive performance.
536.13  BDNF mRNA LEVELS IN THE CHICK OPTIC TECTUM ARE REGULATED BY NEUROTHERMINDS DURING SYNAPTGENESIS. K.-H. HIEZERG* AND Y.-A. BARDE. Dept. of Neurobiochemistry, Max Planck Inst. for Psychiatry, Am Klopferspitz 18a, D-81829 Martinsried, Germany.

We previously reported that BDNF mRNA is expressed in the visual system of the chick embryo. During the earliest time of target encounter, BDNF mRNA levels are regulated in the tectum by electrical activity of the retinatinal ganglion cells (RGC). BDNF mRNA levels increased following optic axonal transection, intratral tracer application, and the GABA A receptor agonist muscimol. Whether the cholinergic contribution in the regulation of the BDNF gene is due to an activity-dependent influence is currently under investigation.

BDNF mRNA is not only detected in the tectum, but also in the retina, and at E11, comparable levels are observed. Using BDNF-peptide antibodies, we observed a BDNF-like immunoreactivity in the retina, but not in the tectum. The most prominent expression was in the ganglion cell layer, but not all ganglion cells were labeled. Suprisingly, following optic axal transection, the levels of this BDNF-like immunoreactivity did not seem to be dramatically reduced when compared to the control retina. We are currently investigating whether BDNF is actually synthesized in RGC or taken up from their surrounding cells in the retina.

536.15  SIGNALS THAT INDUCE EXPRESSION OF LEUKEMIA INHIBITORY FACTOR (LIF) IN RAT SUPERIOR CEREBRAL GANGLION (SCG) AND THEIR EFFECTS AFTER AXONAL TRANSECTION. Y. Sun, S. C. Lardis and R. E. Zigmond. Department of Neurosciences, School of Medicine, Case Western Reserve University, Cleveland, OH 44106.

We previously have shown that LIF contributes to the axonally induced neurotrophic peptide expression in rat and mouse superior cervical ganglia (SCG). The expression of LIF itself is also induced in SCG with 2h after axotomy or explantation. The initial time course of LIF mRNA induction (from 30min to 6h after nerve axotomy) is similar in vivo and in vitro, suggesting that the two systems possess common early LIF-inducing signals after axonal transection. However, there is a difference in the level of LIF mRNA levels between the two systems. LIF mRNA levels start to decline 12h after axotomy in vivo, while they remain elevated until 48h in culture. To examine the early signals responsible for the rapid induction of LIF mRNA after nerve transection, we used short term explant cultures. Defined medium conditioned for 30min by freshly dissected adult rat SCG (SCM) contain LIF mRNA, similar peptide expression when used to direct isolated neuronal SCG cultures with non-neuronal cells. Like SCM, defined medium conditioned by portions of the three major nerve trunks of the SCG for 3h (NCM) also included LIF mRNA levels in dissociated SCG cultures. Further, LIF mRNA induction was observed in these nerve pieces themselves, suggesting that Schwann cells along the axons are the sources of LIF production after nerve lesion. The LIF-inducing activity of both SCM and NCM was present in a >10% fraction and its appearance did not require new protein synthesis. SCM and NCM induced peptide expression in SCG of normal, but not LIF-deficient, mice, suggesting that the effect of SCM and NCM on peptide expression is mediated by LIF. Based on these observations, we hypothesize that when SCG are axotomized, an existing molecule is activated and/or released by transected axons and possibly by axonotomized neuronal cells as well. This molecule in turn causes local Schwann/sensitel cells to produce LIF which participates in regulating neurotrophic peptide expression in SCG neurons.

536.16  INCREASES IN VASOACTIVE INTESTINAL PEPTIDE (VIP) LEVELS IN CULTURED RAT SUPERIOR CEREBRAL GANGLION (SCG) CAN BE REDUCED BY AN ADENYLATE CYCLASE INHIBITOR. R. E. Mohler* and R. E. Zigmond. Department of Neurosciences, Case Western Reserve University, School of Medicine, Cleveland, OH 44106.

The expression of a number of neurotransmitter changes after axonal injury, possibly in the shift in the priorities of these neurons from synaptic transmission to maintenance and regeneration. For example, VIP is normally present in extrinsic trophic and trophic modulatory functions for rat SCG but levels of this peptide increase dramatically after SCG are placed into explant culture. The gene that encodes for VIP contains a cAMP response element that is required for cAMP-regulated transcription. Ganglionic VIP levels are relatively low under normal conditions and do not significantly change in short-term cultures. Previously, we have shown that agents which can affect intracellular calcium levels (for Example, VIP-soluble and quinolnol) are also capable of increasing VIP-like immunoreactivity. We now demonstrate that the increases in VIP levels produced by the addition of cAMP-dependent agents to cultured SCG can be prevented by the addition of an adenylyl cyclase inhibitor, 2'-5'-dideoxyadenosine (DDA). DDA (1 mM) reduced elevations in both CAMP and VIP levels by greater than 60% in SCG cultured in medium containing cAMP-depleting agents or in control medium alone. Histological examination was performed to determine if DDA adversely affected the health of the neurons. No apparent differences in neuronal morphology were found between SCG cultured in control medium or in medium containing DDA for 48 h. An increase in the concentration of DDA to 2 or 6 mM was effective in reducing VIP levels measured in the presence of cAMP-depleting agents by 85% or 95%, respectively. These data support the observations that VIP levels in sympathetic neurons can be regulated by alterations in cAMP levels. Furthermore, they suggest that the increase in VIP that occurs by placement of the SCG into culture with control medium is dependent on the maintenance of basal cAMP levels.

536.17  CHANGES IN GPA LEVELS IN CILIARY GANGLION TARGETS DURING AND AFTER THE CELL DEATH PHASE. P. M. Acosta and H. Y. J. H. Ch. Nathl. Department of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR 97201.

During normal development of the avian ciliary ganglion, the neurons become dependent upon their targets in the eye for survival. The process begins after the birth and continues until E14, by which time half of the original neurons present have died off. Production by the targets of specific neurotrophic factors may mediate the level of neurons which ultimately survive. Growth promoting activity (GPA), a 21 K protein originally purified from adult chicken sciatic nerves and E15 chick eyes, is one such candidate trophic factor. In order to determine what role GPA plays in ciliary ganglion survival, the cellular location and timing of GPA expression in ciliary target was investigated by immunocytochemical, biochemical, and western blot analysis. Antibodies prepared to a purified and highly active form of recombinant GPA detected a 21.6 K protein in chordal-rectum extract on western blots. The intensity of the bands extrapolated to the neurogenic age. Immunocytochemically, the staining of isolated chordal layers revealed that GPA immunoreactivity was localized to smooth muscle cells, and in the anterior portion of the eye. GPA-like immunoreactivity was localized to muscle fibers of the ciliary body. Both of these cell populations are normal targets of CG neurons. GPA expression was first detectable at E10, and increased through the end of the cell death phase at E14. GPA expression continued to increase in the cell phase of the eye, possibly indicating a continued dependence of the neurons for GPA. To test this possibility we are performing neuronal survival assays on various age ganglia to determine the age dependence of the GPA. We are also comparing GPA-like biological activity before, during, and after the cell death phase in an effort to correlate GPA levels with neurotrophic activity in target extracts. Supported by NS25787.

536.18  KOADEIC ACID POTENTIATES AND GENBINEST INHIBITS THE PLATELET-ACTIVATING FACTOR (PAF) ENHANCEMENT OF INDUCIBLE PROSTAGLANDIN SYNTHASE. P.K. Mukherjee, D.J. Smith and N.G. Bazen*. LSU Neuroscience Center, LSU Medical Center, New Orleans, LA 70112.

PAF accumulates in brain during ischemia and convulsions. TS10/PGS-2 is a primary response gene encoding the inducible form of prostaglandin synthase. In the presence of retinoic acid, a PAF-dependent (1-50 nm) activation of luciferase reporter constructs driven by regulatory regions of the TS10/PGS-2 gene transfected into the neuroblastoma cell, NG 10-15 was observed (N. Bazen et al., Proc. Nat. Acad. Sci., in press). Here we report that when DOTAP is used for transfection of TS10/PGS-2 promoter luciferase constructs, PAF elicits a 5- to 10-fold activation in neuroblastoma cells and CV-1 cells. Deletion studies restrict the major PAF cis-acting response element of the TS10/PGS-2 gene to a 70-nucleotide sequence as an intracellular inducer of TS10/PGS-2 expression. Okadec acid (10 ng/ml) elicited a 3-fold potentiation of the PAF effect at 1000 ng/ml, the other hand, inhibits the PAF-induced effect. These results strongly suggest that, in the intracellular pathway of signal transduction between the membrane-dense lipid mediator PAF and the modulation of gene expression of TS10/PGS-2, there is a necessary step that comprises protein tyrosine kinase-protein tyrosine phosphatase. These regulatory events may play a role in neural plasticity responses such as long-term potentiation and epileptogenesis (NINDS NS23002).

We have previously reported that neurotrophin (NGF, BDNF, NT-3 and NT-4/5) mRNA expression is limited to developing and adult optic nerve. Developmental decreases in trkB and especially in trkC expression were also shown by reverse transcriptase-polymerase chain reaction (RT-PCR) using primers that amplified the extracellular domains (Elkebess et al. 1993, Abstr. Soc. Neurosci., 19, 251). To investigate whether mRNA levels detected by RT-PCR are sufficient for the synthesis of neurotrophin and tk proteins, and to determine whether glia express full-length trk receptors, we have performed immunocytochemical studies utilizing antibodies specific for neurotrophins and for the extracellular or intracellular domains of trkB, trkC and trkD (kindly provided by Dr. D. Kaplan, NCI, Frederick, MD).

BDNF, NT-3 and NT-4/5-like immunoreactivities were detected in glia of developing and adult optic nerve. Antibodies to the extracellular and intracellular domains of trkD also labeled cells in the optic nerve, suggesting that glia express full-length forms of the receptors. These findings were confirmed by RT-PCR, using primers that amplified the intracellular trkD domains. The number of cells expressing trkB and trkC in the postnatal day 10 optic nerve appeared to be higher than in the adult, supporting our previous findings. Our studies suggest that neurotrophins are elaborated by optic nerve glia and that responsiveness in glial cells may be mediated through the full-length receptor. Further studies are in progress to determine which glial subtypes express neurotrophins and trks (This work was supported by NIH grant PO1 HD 23315-06A1).

357.3 NERVE GROWTH FACTOR RECEPTOR GENE EXPRESSION IN HUMAN BLOOD. B.D. Kittur*, I. Song, H. Endo, W. Adler. National Institute of Aging, 2940 Eastern Avenue, Baltimore, MD 21224 (further address at New Point VA Medical Center, Perry Point, MD 21902).

Nerve growth factor modulates the immune function as a consequence of binding to the nerve growth factor receptor (NGF-R). Therefore, we hypothesized that NGF-R will be made by the immune system in the peripheral blood lymphocytes. Northern blot analysis of mRNA from human peripheral blood lymphocytes was performed using cDNA probe for low affinity - NGF-R (p75 NGF-R). We show that p75 NGF-R mRNA is present in human peripheral lymphocytes and expressed maximal levels at 14 hours after stimulation with phorbomethyltin. Also, we shows that p75 NGF-R did not change with the aging process in peripheral blood lymphocytes in human volunteers.

357.5 HIGH MOLECULAR WEIGHT NGF-LIKE ACTIVITY IN THE RAT PITUITARY. S. Soinila*, J. Lakshmanan. Dept. Anatomy, PO Box 9, 00014 University of Helsinki, Finland.

We previously reported evidence for NGF immunoreactivity and biological activity in the rat pituitary gland (PG). Since the biological properties of the PG NGF differed from those reported for the rat iris muscle, we examined the molecular properties of the rat PG NGF-like material and compared it with those found in the mouse peripheral sympathetic ganglia (PG). Homogenized PG tissue was subjected to immunoblotting using antisera against mouse 8-NGF. For bioassay studies, PG homogenate supernatants were subjected to ultrafiltration and fractions with MW > 30 kD were tested for neurite outgrowth response from newborn superior cervical ganglion explants. Mouse 8-NGF antisera identified two proteins with MW 53 kD and 73 kD. The 13 kD band was absent in the PG. PG ultrafiltrates containing proteins of MW > 30 kD produced neurite outgrowth from newborn ganglia. Based on our data, we suggest that 53 kD and 73 kD NGF prohormones and that they represent the post-translationally modified products of a short and long NGF prohormone transcripts, respectively.

357.4 DIFFERENTIAL GENE EXPRESSION OF TROPHIC FACTORS BETWEEN EMBRYO AND EXTRAEMBRYONIC MEMBRANES IN CULTURED HUMAN PLACENTAL CELLS. W. Glazer*, D. E. Brennan, N. Jain, and J. M. Hill. Lab. of Dev. Neuroendocr., NICHD, NIH, Bethesda, MD, 20892

Whole mouse embryos cultured have been used to examine the effects of trophic factors on embryonic growth and development in the absence of maternal influences. Exogenous application of vasopressin internal peptide has been shown to increase relative size, protein and DNA content, and aminopeptidases activity (Amer. J. Anat., 1993, 362:155-158). In an effort to characterize the underlying trophic milieus in which these embryos grow, RT-PCR techniques were used to determine the presence and relative amounts of mRNA for known neurotrophic and hormonal factors. Mouse embryos (20-24 somites), with intact extraembryonic membranes, were cultured in 75% human serum and 25% rat serum for 6 hours. At the end of this time, embryos were detached from the membranes, and total RNA was isolated from each embryo and corresponding membranes separately. Equal amounts of RNA were reverse transcribed, and these DNA products used as templates in PCR reactions. Specific PCR primers were designed to detect the following mouse mRNA: basic brain-derived growth factor (BDNF), leukemia inhibitory factor (LIF), transforming growth factor β (TGFβ), basic fibroblast growth factor (bFGF) and insulin-like growth factor 2 (IGF-2). Each primer pair gave a single band of the expected size. There was no difference in actin relative to total RNA between embryos and membranes. Although present in both, there was no significant difference in IGF-2 gene expression relative to actin or total RNA between embryos and membranes. TGFβ was also present in both, and tended to be more abundant in membranes, but the difference was not significant. NFG and BDNF were both significantly higher (> 50 fold) in embryos than in membranes relative to actin and total RNA. LIF was significantly higher (> 20 fold) in membranes than in embryos relative to actin.

(Restricted research supported by the Canadian MRC)


The brainstem locus ceruleus (LoC) participates in learning, memory, and other behavioral functions through its regulatory effects on input responsivity of its target neurons, stimulation of metabolic processes, and interactions with other neurotransmitter systems including the septohippocampal cholinergic system. In the rat, some LoC neurons express BDNF mRNA, but not NGF, whereas in humans, BDNF mRNA is found in the LoC, but NGF is not. Further, the LoC cells of rats are similar to LoC cells with increased number of TH+ neurons. In humans, loss of LoC neurons occurs during aging and is exaggerated in Alzheimer's disease (AD). We examined BDNF mRNA expression in noradrenergic neurons of the human LoC using in situ hybridization with an S32-labeled riboprobe synthesized from a 477 bp EcoRI/PstI fragment subcloned from a human BDNF cDNA (gift of K. Jones and L. Reichard). Fresh frozen brainstem were obtained at autopsy from 3 adult human brains (1 AD, 1 dementia with gliosis, 1 control) and from a 20-week fetal human specimen following elective abortion. In adult hybridization was performed on 20μm cryosections using 2 pmol/ml labeled antisense or sense riboprobe and tissues examined by film and emulsion autoradiography. Expression of BDNF mRNA was found in noradrenergic neurons of rats. No expression was found when BDNF mRNA was examined in noradrenergic neurons of all 3 adult human brains (1 AD, 1 dementia with gliosis, 1 control) and from a 20-week fetal human specimen following elective abortion. These results suggest that BDNF in the human LoC may have a functional autocrine or paracrine role during development of the human LoC. Supported by grants from the Alzheimer's Association [S Bornstein PI] and NIA (UW ADRC [C Martin PI], and by the UW Fetal Tissue Program [A Fastel PI].

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537.7
TRANSIENT EXPRESSION OF BDNF mRNA IN RAT LOCUS COERULEUS FOLLOWING ACUTE 6-OHDA LESIONS OF THE NIGROSTRIATAL PATHWAY. S. Numan* and K.B. Serogy. Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536.

In vitro studies indicate that certain members of the neurotrophin family of trophic factors can support the survival of, and are neuroprotective for, populations of catecholaminergic neurons. In the present study, the expression of various neurotrophic factors in rat brain and neurotrophin-3 (NT-3) mRNAs in neurons of the locus coeruleus was examined in vivo following acute lesions. The animals received a single injection of 6-OHDA (10 mg/kg; i.p.) or vehicle by following survival times of 4 hr, 8 hr, 16 hr, 24 hr, 3 days, 4 days and 8 days. Cryostat-prepared tissue sections through the locus coeruleus were subsequently processed for the in situ hybridization localization of BDNF, NT-3 and TH mRNAs using 35S-labeled cRNA probes. BDNF mRNA was barely detectable in locus coeruleus in normal, control rats and in vehicle untreated rats. After 6 hr or 16 hr, a substantial increase in hybridization density for BDNF mRNA was observed in locus coeruleus which peaked at 24 hrs and gradually returned to control levels by 8 days post-injection. TH mRNA in the nucleus exhibited a similar transient elevation, in agreement with previous reports. The data indicate that depletion of catecholamines induces a transient increase in BDNF mRNA expression in locus coeruleus neurons and raise the possibility of BDNF involvement in neurological disorders associated with the locus coeruleus, such as depression. Supported by the National Parkinson Foundation and the Scottish Rite Schizophrenia Research Program.

537.8
INCREASED EXPRESSION OF BDNF mRNA IN ADULT VENTRAL MIDBRAIN FOLLOWING ACUTE 6-OHDA LESIONS OF THE NIGROSTRIATAL PATHWAY. S. Numan* and K.B. Serogy. Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536.

In vitro studies have demonstrated that BDNF and NT-3 support the survival of fetal rat mesencephalic dopamine neurons. Furthermore, several studies have provided evidence that BDNF and neurotrophin-3 (NT-3) mRNAs in dystrophic neurons were expressed in response to a variety of insults. In the present study, we have examined the expression of BDNF and NT-3 mRNAs by cultured neurons for cultured dopaminergic neurons undergoing various toxic insults. In vitro, adult dopaminergic neurons, which express BDNF and NT-3 mRNAs may initially respond to various toxic insults by altering the expression of neurotrophin mRNAs. To examine this possibility, adult rats received unilateral injection of 6-OHDA or vehicle into the ascending medial forebrain bundle. Following various survival periods (8, 16, 48 and 120 hrs), in situ hybridization of 35S-labeled cRNA probes for BDNF, NT-3 and TH mRNAs was performed in midbrain sections. After 8, 16 and 48 hrs, an increase in the hybridization density for BDNF mRNA was observed in the substantia nigra and ventral segmental area ipsilateral to the 6-OHDA injection, as compared to the uninjected control side. In contrast, no alterations in the hybridization density for NT-3 or TH mRNAs were observed at these time points. After a 120 hr survival period, there was a decreased hybridization density for TH, BDNF, and NT-3 mRNAs in the ventral midbrain ipsilateral to the 6-OHDA injection, as compared to the control side. No alterations in the hybridization density for BDNF, NT-3 or TH mRNAs were observed in the ventral midbrain of rats that received unilateral injections of vehicle. These data indicate that there was an early transient response by BDNF mRNA in the ventral midbrain to the neurotoxin 6-OHDA, which may suggest a role in vivo for this neurotrophin in neuronal protection. Supported by the National Parkinson Foundation and the Scottish Rite Schizophrenia Research Program.

537.9

We examined the expression of endogenous BDNF in adult rat brains using a BDNF specific antibody by immunohistochemistry. We found distinct BDNF immunoreactivity (IR) in many structures which are known to be BDNF mRNA positive from previous studies. These structures included neocortex, piriform cortex, hippocampal formation, claustrum, supramammillary region and other thalamic nuclei, some hypothalamic nuclei, substantia nigra, and neurons in many brain stem structures. In the hippocampal formation, very heavy BDNF IR was found in the granular layer of dentate gyrus and pyramidal and radiatum layers of CA3 and CA4, but not in CA1 and CA2 regions of hippocampus. However, with kainic acid treatment (4p), which is known to upregulate BDNF mRNA, BDNF IR was readily detected in CA1 and CA2 regions. Staining was also seen in areas not previously reported as BDNF mRNA positive. These included the bed nucleus of stria terminalis, dorsal endopiriform nucleus, lateral septum, medial preoptic nucleus, olivary pretectal nucleus, lateral paragiganto-cellular nucleus, and nucleus ambiguus. The results of this study suggest BDNF may play a broad role in the adult central nervous system.

537.11
STRUCTURE, EXPRESSION AND FUNCTION OF RAT GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF). M. Trojan1, M. Pedel1, L. Areval1, T. Timmus2, N. Lindvall3 and E. Ljungberg1. 1Laboratory of Molecular Neurobiology, MBB and 2Department of Clinical Neurobiology, Karolinska Institute, S-17177 Stockholm, Sweden.

A cDNA encoding rat GDNF was cloned by RT-PCR from P1 rat brain RNA. An alternatively spliced variant (termed GDNFp), similar to that described by Schaer et al. (1993), with a 78 bp deletion in the prepro region was also cloned. Both mRNAs were expressed in developing striatum, cerebellum and some peripheral organs as assessed by in situ hybridization. GDNF mRNA expression was developmentally regulated and appeared to be altered by neuronal survival. The cellular localization of GDNF mRNA expression during rat development is being studied by in situ hybridization.

Western blotting and immunoprecipitation analysis with anti-GDNF antibodies generated against predicted loop regions of the molecule showed that the detection decreased with age. This decrease was correlated with GDNF protein produced by transiently transfected COS cells. Recombinant GDNF produced in baculovirus-infected insect cells was correctly processed and glycosylated. This protein purified to homogeneity promoted survival and neurite outgrowth from embryonic sympathetic ganglia with a time-course and morphology clearly different from nerve growth factor, suggesting activation of distinct signalling pathways. Genetically engineered fibroblast cell lines producing high levels of GDNF are currently being used to investigate the function of GDNF in vivo.

537.12

GDNF is a newly described member of the TGF-b family isolated from the rat glial tumor Oligo line. GDNF promotes survival, dopamine uptake, and neurite outgrowth of embryonic dopaminergic (DA) neurons in vitro. We have used a semi-quantitative RT-PCR with primers specific to GDNF to study the development of GDNF expression in CNS and peripheral tissues of embryonic rat on gestational days E13.5 and E18, neonatal rat on postnatal days P0 and P10 and adult rat. GDNF mRNA is expressed in all major regions of the CNS; however, the level of expression is both region and age dependent. The highest levels of GDNF mRNA are expressed in P0 spinal cord and in P0-10 striatum. The lower levels are expressed in brainstem (including ventral mesencephalon, which contains the substantia nigra), cerebellum, diencephalon and telencephalon. GDNF mRNA is also expressed in E10-12 limbs, E0-12 heart, and E12-16 eye. GDNF expression is higher than in brain, particularly in embryonic limb bud, kidney and gut; neonatal kidney, gut, lung and testis; and adult lung, testis, and heart. GDNF mRNA is also present in the head, torso and chorion/umbilical of the E11.5 embryo.

These observations suggest that GDNF may be a target derived and/or locally acting neurotrophic factor for DA neurons of the substantia nigra, as well as for other classes of CNS neurons. Moreover, GDNF may play an early role as a neurotrophic factor in the PNS or serve as a growth factor for non-neuronal cells. (Supported by the Markey Charitable Trust & NIH grant ES01347). 1 L.-F. H. Liu et al., Science 260, 1130-2 (1993).
ISOLATION OF MULTIPLE GDNF TRANSCRIPTS FROM THE C57BL/6J GLIOMA CELL LINE. M. D. Spence, A. Y. Deutch, and E. Lolis. Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

Glioma cell line-derived neurotrophic factor (GDNF) is a newly discovered member of the transforming growth factor-β superfamily which exhibits neurotrophic effects on midbrain dopamine neurons both in vitro and in vivo. GDNF was originally isolated from the B49 glioma cell line. We have analyzed two other glioma cell lines, the rat C6 glioma and the human H6863 glioma, for expression of GDNF. Polyadenylated mRNA was isolated from these cell lines and subjected to RT-PCR amplification for first strand cDNA synthesis. Polymerase chain reaction was used with primers against regions near the initiating methionine and terminal stop codon to amplify the sequence. Two bands were seen from C6 cells when the PCR reaction products were analyzed by agarose gel electrophoresis and visualized with ethidium bromide. In contrast, no bands were present in the H6863 PCR reaction.

These results indicate that expression of GDNF is not limited to B49 cells but also occur in C6 cells. In addition, the C6 cells express two GDNF transcripts, which has not been previously reported. Further characterization of these transcripts is in progress.

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S88.1 THE NEUROTROPHIC SYSTEM OF BASIC FGF OVEREXPRESSING TRANSGENIC MICE. F. Ekenstein*, C. Hicks
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Fibroblast growth factors (FGFs) in vitro exhibit a wide variety of neurotrophic and mitogenic effects. Nine members of the FGF multigene family are currently known. The adult central nervous system contains very high levels of acidic and basic FGF (aFGF and bFGF), molecules that lack hydrophobic signal peptide sequences and are not efficiently released from normal healthy cells. Consistent with these observations is our hypothesis that aFGF and bFGF function as initiators of injury responses in CNS.

In order to test this hypothesis we have obtained (a generous gift from Drs. D. Coffin and R. Flikkervis) transgenic mice that overexpress human NGF. Initial characterization of these animals showed a highly significant increase in bFGF levels in the CNS of transgenic animals. No gross morphological abnormalities were detected in the brains of these animals, and highly preliminary experiments did not show marked changes in the immunohistochemical distribution of neuropeptides and neurotransmitter synthesizing enzymes.

S88.2 REGULATION OF RAT BDNF GENE PROMOTERS IN TRANSGENIC MICE. T. Timmers*, U. Lendvai*, H. Munkwitz, E. Aarburg and M. Heilstett, Lab. of Molecular Neurology and Dept. of Developmental Biology, Karolinska Institute, 17177 Stockholm, Sweden.

The rat brain-derived neurotrophic factor (BDNF) gene consists of four 5' exons linked to separate promoters and one 3' exon encoding the prepro-BDNF protein. Transgenic mice have been generated, that express bacterial chloramphenicol acetyl transferase (CAT) under the control of six different promoter constructs of the BDNF gene. When fused separately to bacterial CAT gene the upstream regions of the 5' exons of rat BDNF gene were linked to the transplant expression in tissues that partially overlapped with the endogenous sites of particular BDNF promoter activities. High-level expression of reporter gene in correct neuronal populations of BDNF intron-exon splice junctions and 3'UTR in the constructs. The genomic regions responsible for the in vivo upregulation of BDNF expression after kainic acid-induced seizures and KCI-induced spreading depression were mapped. We also report that 5.5 kb of BDNF promoter IV sequence directs axotomy-induced expression of reporter gene in the sciatric nerve of transgenic mice in a manner similar to the regulation of endogenous BDNF mRNA in the same paradigm. These BDNF promoter constructs provide useful tools for targeted expression of neurotrophic factors to study their function and regulation in central and peripheral nervous system.

S88.3 A CAUCAL responsive PROMOTER IN THE RAT BDNF GENE. J.E. Bishop*, G.L. McDonald and B.D. May, Department of Pharmacology Unit, Experimental Therapeutics Branch, NINDS, and Department of Physiology, USUHS, Bethesda, MD 20882.

Transcription of the rat brain-derived neurotrophic factor (BDNF) gene results in multiple transcripts containing at least five alternate first exons that are separately spliced to a common protein coding exon. Since Ca2+ is a prominent second messenger involved in the regulation of BDNF expression, rat C6 glioma cells were incubated with BDNF, exposed to the Ca2+ ionophore A23187 and BDNF mRNA levels were measured by reverse transcription PCR. The cells were incubated with A23187 (5 X 10-5 M) or vehicle for 1 hour, rinsed, incubated another 3 hours in culture medium and total RNA was extracted. PCR results indicated that BDNF coding exon containing transcripts were elevated approximately 9-fold compared with vehicle treated controls. Inclusion of the RNA polymerase inhibitor actinomycin D (10 uM) in culture medium led to significant decrease in both basal and Ca2+ stimulated BDNF mRNA concentrations indicating that, in C6 cells, BDNF expression is regulated primarily at the level of transcription. To identify which of the multiple promoters in the BDNF gene is transactivated by increased calcium, the effects of A23187 on the expression of each of the alternate 5' exons was investigated. Of the three alternate 5' exons expressed in C6 cells, only one (exon 1E) increased significantly (approximately 4-fold) which is less than the response of the common coding exon measured in the same samples. Additional calcium-responsive promoters directing expression of the BDNF gene not detected in the present investigation may account for this observation.


The rat brain-derived neurotrophic factor (BDNF) gene has been shown to contain four short 5' exons (Exons III-V) and one 3' exon (Exon V) that encodes the mature BDNF protein. BDNF mRNA transcripts containing the different 5' exons can be differentially induced in brain following seizure although the mechanisms by which this occurs have yet to be elucidated. In the present study, the effect of anisomycin, a protein synthesis inhibitor, on the expression of Exon I and III mRNAs following a stimulated epileptiform afterdischarge of hippocampal slices was analyzed in adult rats. Two groups were used: 1) single hippocampal afterdischarge (AD) via perforant path stimulation (10 Hz, 4-6 sec) and sacrificed 1.5 hrs post-AD; 2 anisomycin (150 uM/kg, s.c.) injected 30 min prior to and 1 min after following AD, and sacrificed 1.5 hrs post-AD; 3) 2 injections of anisomycin 1 hr apart and sacrificed 1 hr after last injection; 4) naive controls. Changes in BDNF exon mRNA content in the granule cells were assessed by in situ hybridization using 35S-labeled cDNAs. Following AD, Exon I mRNA was increased 10 to 30-fold above control levels in the granule cells. This effect was reduced by 82% with anisomycin. Like Exon I, Exon III mRNA was markedly increased following AD to approximately 7-fold above control levels. However, the effect of AD on Exon III expression was not reduced by anisomycin. Anisomycin alone had no effect on either Exon I or III. The present results show that Exon III expression can be increased in the presence of protein synthesis inhibitors suggesting that this exon, as opposed to Exon I, is directly induced by physiological activity without intervening protein synthesis (i.e., as an immediate-early gene). The presence of distinct mechanisms regulating the transcription of the 5' exons within one cell type suggests each may be induced under different circumstances. Supported by NS28748 to C. G., EPA & Abbott Labs award to J. L. and Mayo Foundation to P. I.

S88.5 Identification of an Inducible Promoter in the Nerve Growth Factor Gene that Initiates Transcription at exon 5. MM Racker, P. J. Mason, M. Johnson, B. V. Siegel*, M. Lindik, Marion Merrell Dow Research Institute, Cincinnati, OH 45215.

NGF has been demonstrated to facilitate neurite outgrowth, rescue neurons from injury, and prevent programmed cell death. However, the therapeutic potential of NGF is limited by pharmacological difficulties common to many multimodal pharmaceutical agents. This difficulty can be circumvented by identifying compounds which facilitate endogenous transcription of NGF in the brain. Thus, we sought to determine the site of all pharmacologically inducible promoters in the NGF gene using a differential analysis based on semi-quantitative reverse transcription PCR. L929 cells were serum deprived and NGF was induced by treatment with PMA and calcitriol (1,25-dihydroxy-vitamin D3). Of the 4 major transcripts previously identified in mouse (Mot Cell Biol, 7:3057,67), a 2.5 - 4 fold increase in transcripts initiated at exon 1 was noted in cDNA from cells induced with PMA and calcitriol, confirming the previous demonstration of a promoter region near exon 1 (Mot Brain Res, 15: 67,50). In addition, we also noted a 3 - fold increase in cDNA transcripts initiated at exon 3, suggesting a second, previously unidentified inducible promoter in this region. Sequence analysis revealed a consensus HRE in the 3' region of exon 3 which contains increased esterase forming sequences. In conclusion, these studies demonstrate a second inducible promoter in the NGF gene near exon 3 that could be targeted for therapeutic intervention.

S88.6 PRODUCTION AND PROCESSING OF THE NGF FAMILY OF NEUROTROPHIC FACTORS. L. Llamas*, W. Zhu, Q. Luo, L. Stasiak and M. Fahmy, Department of Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

The neurotrophic factors NGF, BDNF and NT-3 are translated as pro-pre peptides which require proteolytic processing at dibasic amino acid residues to attain their mature, biologically active forms. Little is known about the processing of the pre-pro forms of the neurotrophins. The ability of fibroblast NGF, and the presence of related enzymes in human brain, suggest these serine proteases may play a role in neurotrophic factor processing. The study of neurotrophic factor processing has been hindered by the lack of sufficient substrates for analysis. We report here the production of quantities of pre-pro and mature neurotrophic factors sufficient for processing studies.

Proteasome were constructed expressing cDNAs for NGF and NT-3 as well as PC2, a member of the prohormone convertase family of proteases related to furin. The resultant recombinant viruses were used to infect S9 fibro cells, and both mature and pro-pro trophic factors were identified on SDS-PAGE gels and analyzed by Western Blotting. The biological activities of the neurotrophins were confirmed in neurite outgrowth assays using neonatal mouse SCG and DRG neuronal cultures. Expression of PC2 was confirmed by Western blotting and protease assays using synthetic substrates.

Coinfections were carried out using NGF and NT-3 recombinant virus along with PC2 retrovirus. This could induce neurotrophic factor in cell lines. The extent to which PC2 could enhance processing was determined by photographic analysis of the bands from the Western Blots. The NGF and PC2 cotransfected supernatants were more proficient in processing the trophic factor than the control infection, whereas NT-3 + PC2 cotransfected supernatants showed about a three fold increase in mature NT-3. (Supported by NIH and MRC)
S038.7  
**Beta-Nerve Growth Factor (NGF) is an artifact.** J. Lakshmanan*, S. Sridevi*, K. Pesonen†, T. Lahelma†, A. Baker*, J. Perheentupa* and D. A. Fiddler‡.  
CLA Medical Center, CA, USA and University of Helsinki, Helsinki, Finland.  
We previously reported the absence of NGF in normal adult mouse sera of both sexes and liver (†) and in sera of adult male markedly exercised mice (115-490 ng/ml) [Am. J. Physiol. 250: E386-E392 1986]. We have now examined the molecular species of serum NGF both by immunoblotting and column chromatography (CM-52). All sera (250 µl) of mouse sera were immunoprecipitated with beta-NGF antiserum and the precipitates analyzed by immunoblotting. Similar volumes of sera were subjected to Sephadex G-100 chromatography. Immunocomplexes were analyzed by Western blotting with beta-NGF-R IA. Immunoblotting analysis revealed the presence of a 53 kDa NGF protein but no 13 kDa NGF immunoreactive protein in sera of either normal or aggressive male mice. The radioactive intensity of 53 kDa NGF protein was several fold greater in aggressive mouse sera compared to normal mouse sera. By chromatographic analysis, the NGF-immunoreactivity of normal male mouse sera eluted as a single peak that appeared in the void volume while that of aggressive mice eluted in two distinctive peaks. The first appeared in the void volume (as in normal mice sera) and the second corresponded to the elution profile of 13 kDa NGF. The later peak accounted for more than 90% of total immunoreactivity. Both aggressive mouse sera and SMG-53 kDa NGF stimulated neurite outgrowth from chick embryo dorsal root ganglion explants. The absence of 13 kDa NGF in normal mouse sera immunoprecipitates and in aggressive serum samples subjected to Sephadex chromatography suggest that the 13 kDa NGF is an artificial proteolytic fragment of 53 kDa NGF generated in vitro during chromatography.

S038.8  
**IDENTIFICATION OF RNA BINDING PROTEINS WHICH MAY CONTROL NERVE GROWTH FACTOR mRNA STABILITY.** B. Tsang*, S.P. Palemnik* and B.C. Wise.  
Fidia-Georgetown Institute for the Neurological and Department of Pharmacology, Georgetown University, Washington, D.C. 20007.  
Our previous studies have shown that interleukin-1 and okadacid acid, a phosphatase inhibitor, increase the level of the full-length nerve growth factor (mRNA) in primary cultures of cortical astrocytes. The 3'-untranslated region (UTR) of the NGF mRNA contains an AU nucleotide rich region which, similar to other short-lived mRNAs, might modulate its rate of degradation. Using Northern blotting analysis, we have now identified a protein with a MW of about 140 kDa which binds to a radiolabeled RNA probe containing the AU-rich domain in the 3'UTR of the eukaryic mRNA. RNA binding activity was observed using a full-length NGF RNA probe, but was not seen using a probe lacking the 3'- UTR or using a β-actin RNA probe. The binding activity or amount of this protein was increased in astrocyte cultures treated with okadacid acid (5-30 μM). Maximal induction of binding activity was seen following 9 h of treatment at the maximal okadacid acid concentration tested. Under these conditions NGF mRNA content was also elevated by about 5-fold compared to unstimulated astrocytes. The RNA binding activity was abolished by treating astroglial cells with trypsin, and the induction of the RNA binding protein by okadacid acid was prevented by cotreatment with cycloheximide (10 μg/ml) indicating that okadacid stimulates the synthesis of this putative modulator of NGF mRNA degradation. Finally, the induction of this binding activity by okadacid acid is associated with an increase (4-fold) in the 5' end of the NGF mRNA. Transfection of cell lines with expression vectors containing the full-length NGF cDNA or NGF cDNA lacking the 3'UTR are underway to study the role of the 3'-UTR and the putative modulator in the control of NGF mRNA degradation.

S038.9  
**PROCESSING OF NGF, BDNF, AND NT-3 PRECURSORS BY PROHORMONE CONVERSIONS.** N. G. Seidah*, S. Pareek*, S. Benajma*, J.-L. Campenot†, M. Chretien†, and R. A. Murphy‡.  
Montreal neurologic institute, McGill University and the Clinical Research Institute of Montreal, Montreal, Quebec, CANADA.  
Conversion of prohormones and prohormone proteins into biologically active molecules involves the concerted action of a number of convertases. Furin can process the precursor of mouse B-NGF (LIF-Like 10, Cell Biol, 111, 2855-2859, 1990) but no information is yet available to explain how other members of the neuropoithin family are generated. In this study, we have compared the processing of pro-BDNF and NT-3 by prohormone convertases. Recombinant vaccinia virus vectors were used to co-express the convertases PC1, PC2, and furin together with NGF, BDNF or NT-3 in human colonic mucosa Lovo cells, lacking endogenous furin. Virally infected cells were metabolically labeled with 35S-methionine and conditioned medium was treated with antibodies to NGF which is immunoprecipitated and identified on Western blots using a BDNF-specific peptide polyclonal antibody. Results show that NGF is synthesized in Lovo cells as a 48 kDa precursor (probably glycosylated) and processed into 14.5 (probably glycosylated) and 13.2 kDa products. NGF processing was effectively carried out by furin and no other convertase. NT-3 was processed efficiently by furin with a small amount of processing carried out by PC1 and PC2 as well. BDNF was effectively processed by furin, with some processing by PC1. These studies suggest that the neurotrophins are most efficiently processed by furin, and that other convertases have limited activity as well.

S038.10  
**DETECTION OF PROHORMONE CONVERSIONS IN DEVELOPING AND ADULT MOUSE SUBMANDIBULAR GLANDS.** H. Farhadi, S. Pareek*, M. Menkenkiewicz†, N. G. Seidah*, M. Chretien†, and R. A. Murphy.  
Montreal neurologic institute, McGill University and the Clinical Research Institute of Montreal, Montreal, Quebec, CANADA.  
The processing enzyme(s) responsible for generating mature β-NGF from its precursor at the amino terminal end have not been identified. Prohormone processing enzymes, called convertases, have been implicated in the processing of precursor proteins at pairs of basic and acidic residues that create a signal for recognition by these enzyme complexes. Two laboratories presented at this meeting (Seidah et al., 1994) have shown that at least one member of this family can cleave NGF, BDNF, and NT-3. The aim of this study was to investigate the relationship of NGF and convertases in the mouse submandibular gland. Northern blot analysis and in situ hybridization revealed high levels of mRNA coding for PC1, PC2, PCs and furin in the salivary glands of neonatal and pubescent male mice; levels in NGF containing granular tubule cells of adults are significant but lower than during pubescence. These results were confirmed by immunocytochemistry. Levels of PC1 mRNA and protein were significantly lower than those of the other processing enzymes. Adult female salivary glands expressed only low levels of NGF and processing enzymes. Our results suggest that one or more prohormone convertases may be involved in processing the precursor of NGF in mouse submandibular glands.

S038.11  
**RETROGRADE TRANSPORT AND SIGNALING MECHANISMS BY NERVE GROWTH FACTOR AND LEUKEMIA INHIBITORY FACTOR IN SYMPATHETIC NEURONS.** D. B. Vege and R. B. Compaan†.  
Department of Anatomy and Cell Biology, University of Alberta, Edmonton, AB T6G 2H7.  
Nerve growth factor (NGF) and leukemia inhibitory factor (LIF) are both retrogradely transported by rat sympathetic neurons in a compartmental culture system. We have further characterized the retrograde transport of 125I-NGF and 125I-LIF. The level of NGF transport appears to be unaffected by the acute presence of LIF. Reciprocally, LIF transport is equivalent whether neurons are supplied with 125I or 200 I. In contrast, pretreating neurons for 6 days with LIF leads to the reduction of both NGF and LIF transport by at least 50%, suggesting that LIF-differentiated neurons may display attenuated NGF-dependent responses. NGF transport levels are not affected by brain derived neurotrophic factor of neurotrophin-3, added concurrently and at concentrations in 100-fold excess of 125I-NGF. Kinetic analysis of NGF transport/processing revealed that following a 5-hr pulse of 125I-NGF to peripheral neurons, most of the radiolabel is transported and released within the first 24 hours. However, cell-associated radiolabel is still present several days following the pulse. Experiments are now being performed to address whether the retrograde transport of NGF or LIF is necessary for transsynaptic changes induced by these signaling ligands.

S038.12  
**ASSAY-DEPENDENT VARIABILITY IN THE CROSS-ACTIVITY OF B-NGF ANTIBODIES WITH RELATED NEUROTROPHINS.** S. Varon* and J.M. Connor.  
UCSD, Department of Biology, La Jolla, CA 92037.  
Previous studies have indicated that antibodies raised against purified β-NGF may cross-react with other neurotrophin family members sharing similar sequence homology, such as BDNF, NT-3 and NT-4. These antibodies generated against any one neurotrophin must, therefore, be examined for potential cross-reactivity with the others. The assay system used to evaluate cross-reactivity is frequently chosen for its convenience. In our B-NGF antibodies, we have found that the choice of the assay system markedly affects cross-reactivity results. In an in vivo assay (following SDS-PAGE), mouse and human NGF were robustly detected by the antibodies while NT-3 and BDNF were virtually undetectable. In a blot-dot assay, the antibodies recognized B-NGF and human BDNF but only detected after fixative treatment of the blot. In a one-site ELISA (with the same antibody for capture and detection), no cross-reactivity with BDNF, NT-3 or NT-45 occurred. When blotted into normal rat (intraocular) injection; 60ng each), all four neurotrophins were recognized by the antibodies. This difference may be attributed to the same extent. In vitro assays with dissociated E8 chick sensory neurons showed that BDNF, NGF, and NT-45 supported cell survival (to varying degrees), but the antibodies could only block the biological activity of NGF. These findings clearly demonstrate that cross-reactivity between NGF antibodies and related molecules is a function of assay system and caution against transferring antibody specificity data across assay systems. Supported by NIHGCs NS-15649.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
NEUROTROPHIC FACTORS: EXPRESSION AND REGULATION III

539.1
INCREASED INSULIN-LIKE GROWTH FACTOR II GENE EXPRESSION IN DIABETIC RAT LUMBAR DORSAL ROOT GANGLIA AND MOTEONUROS. P.J. Lucas*, A.S. Dept and A.J. Yank. The Diabetes Institutes, Eastern Virginia Medical School, Norfolk, VA 23501.

Neuropathy is a major complication of diabetes mellitus and can involve sensory as well as motor neurones. While the neuropathy has been identified, the contributions of a number of pathogenic mechanisms have been extensively studied. The insulin-like growth factors (IGFs) promote neurite outgrowth in vitro and spinal regeneration in vivo. These data coupled with reports of reduced IGF receptor levels suggest the possibility that a decline in IGFR available to neurons contributes to the pathogenesis of diabetic neuropathy. To address this possibility, we examined IGF-II gene expression in lumbar dorsal root ganglia (DRGs) and spinal cords of rats made diabetic by a single dose (5mg/kg, i.p.) of streptozotocin. Age-matched, sham-injected rats served as controls.

After nine weeks of diabetes (blood glucose levels maintained between 200-400mg/dl) the diabetic rats and the age-matched controls were sacrificed and tissues harvested for in situ hybridization with a cRNA probe to IGF-II. Contrary to our expectations, preliminary quantification revealed a three to four-fold increase in IGF-II mRNA levels in lumbar DRGs of diabetic rats as compared to those of normal, non-diabetic rats. Similar changes were observed in lumbar spinal motoneurons.

These observations seem enigmatic in the light of diminished regenerative capacity of sensory and motoneuron axons in diabetic rats. It is possible, however, that a compensatory up-regulation of local IGF-II synthesis by neurons, in response to low serum levels or reduced uptake in diabetics, inhibits axonal regeneration by switching-off the regenerative program normally exhibited by axotomized sensory and motoneurons.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994

539.3
SELECTIVE EXPRESSION OF NEUROTROPHIN-3 mRNA IN MUSCLE SPINDLES OF THE RAT. L.C.V.M. Copray* and N. Brouwer. Department of Medical Physiology, University of Groningen, 9712 KZ Groningen, The Netherlands.

To evaluate the possible involvement of NT-3 in the formation and maintenance of muscle spindles, an in situ hybridization study was set up to analyze the expression of neurotrophin-3 (NT-3) mRNA in muscle spindles from the first embryonic stages of their formation until their mature appearance in adults. Serial, sagittal cryosections of 25 µm were made of 4% paraformaldehyde-fixed intact hindlimbs or dissected hindlimb muscles derived from embryonic (E15-E21), neonatal (P16,P17 and P30) and adult Wistar rats. In situ hybridization for NT-3 mRNA was done in combination with the immunohistological detection of desmin, calcitonin and strioreactive MHC. Starting from the appearance of the first formed intrafusal fiber, i.e. the nuclear bag fiber, at E19, the intramuscular expression of NT-3 mRNA was confined to the intrafusal fibers of the muscle spindles. High levels of NT-3 mRNA were found in the equatorial region of the nuclear bag type intrafusal fibers and remained present in these intrafusal fibers throughout life. During the entire period of muscle formation, examined from E15 on, as well as in mature muscles, no NT-3 mRNA could be detected in extrafusal fibers. The exclusive intramuscular expression of NT-3 mRNA within intrafusal fibers substantiates the involvement of NT-3 in the formation as well as the maintenance of muscle spindles.

539.4

Expression of BDNF, NT-3 and NT-4, was examined in rat skeletal muscle during development (E15-adult) and in adult rat gastrocnemius muscle at different times after transection of the sciatic nerve using quantitative Northern blot analysis of poly A+ RNA and in situ hybridization. At E15, the highest level of expression was found for NT-3 (63pg/gg poly A+ RNA), approximately 20 times greater than that of either BDNF or NT-4. During later development, the levels of all neurotrophins mRNA fell. BDNF and NT-3 mRNA remained low in adulthood, whereas NT-4 mRNA levels increased postnatally, such that adult muscle contained similar quantities of NT-3 and NT-4 mRNA, but very little BDNF message. In situ hybridization of E15 limb bud showed a clear signal for NT-4 only in epimerids, for BDNF mostly in mesenchyme and in some muscle cells, while NT-3 was abundantly expressed in muscle. 24 hours after sciatic nerve lesion, the levels of BDNF and NT-4 mRNA in muscle were reduced considerably. While the amount of NT-4 mRNA remained low, BDNF mRNA levels increased within 2 days after lesion, reaching a 2.6-fold greater than control 14 days after lesion. In contrast, NT-3 mRNA was not affected after nerve injury. Hybridization of transverse sections of denervated muscle with a BDNF riboprobe gave a signal over peripheral nerve. No significant labeling was found in the surrounding muscle tissue. The same riboprobe also labels cells in a distal segment of the sciatic nerve 4 weeks after lesion, suggesting that the source of increased BDNF mRNA production in denervated muscle is Schwann cells in peripheral nerve leading through the muscle.
Distinct regulation of NT-4 mRNA in the skeletal muscle suggests a role for NT-4 in the maintenance of the neuromuscular junction. F. Nandi, L. B. Paradiso, E. Refinetti, V. Rasovskaya, A. M. Johnson, and B. T. Williams. Dept. of Pharmacology and Neurology, U.C. School of Medicine, Los Angeles, Calif. 90024, and Department of Anatomy and Neurobiology, U.C. Irvine, School of Medicine, Irvine, Calif. 92717.

NT-4 expression was increased during postnatal development. The differential regulation of NT-4 mRNA expression suggested that its level may be regulated by motoneuron activity. In agreement with this, identical stimuli caused increased NT-4 mRNA levels in gasserian and soleus muscles in a dose-dependent manner. NT-4 mRNA levels peaked 12 hrs after stimulation and decreased thereafter, reaching basal levels 24 hrs after stimulation. Elevated NT-4 mRNA levels were detected after repeated stimulations. A similar effect could be obtained after direct stimulation of the muscle. In contrast, the mRNA levels of the other neurotrophins were not affected by the electrical stimulation. These observations suggest NT-4 may play a local role in the maintenance of the neuromuscular junction in an activity-dependent manner. This possibility is currently being explored using purified recombinant NT-4 protein and grafts of genetically engineered fibroblast producing NT-4.

An RNA splicing mutation that disrupts the human ciliary neurotrophic factor (CNTF) gene. R. Takahashi, H. Misawa, H. Yokoi, M. Hayashi, T. Komori, A. Inaba, T. Ohtake, and T. Deguchi. Dept. of Neurology, (1), Tokyo Metropolitan Institute of Medical Science, Fuchu City, Tokyo, Department of Neurology (2), Tokyo Metropolitan Neurological Hospital, Tokyo, and Research and Development Center (3), BMJ, Inc., Saiamia, Japan.

Ciliary neurotrophic factor (CNTF) promotes the survival of a variety of neurons. A recent report showed that disruption of the CNTF gene in mice caused motor neuron degeneration. The potential role of CNTF in the survival of motor neurons (Masu et al., 1993). We report a null mutation in the human CNTF gene. The mutated allele shows a G → T transition producing a new splice acceptor site in the removing mRNA species codes for an aberrant protein. Analysis of tissue samples from various genotype subjects and transfected of CNTF minigene into cultured cell demonstrates that the mutated allele expresses only the mutated mRNA species. Mutated CNTF protein is not detected either by immunoblot or immunohistochemical analysis of human tissue as well as any murine tissue. The three simian cells is highly and healthy and neurological disease subjects. Detailed electrophysiological tests on 30- and 64-year-old mutant homogygote reveals no apparent abnormalities. In contrast to the report on mice, our findings indicate that human CNTF deficiency does not induce obvious neurologic dysfunctions.

NEUROTROPHIC FACTORS: EXPRESSION AND REGULATION IV


Gial-derived neurotrophic factor (GDNF) is a recently described neurotrophic factor with trophic activity in mesencephalic dopamine neurons. In rat brain GDNF mRNA expression decreases during postnatal development to very low levels in the adult. In the present study, the influence of lesion- and kainic acid-induced seizures on GDNF mRNA content in adult rat brain was examined. In untreated rats, in situ hybridization to GDNF mRNA revealed only faint signal in the anterior thalamus but was not detected in hippocampus. In association with hilus lesion-induced limbic seizures (which recur from 2 to 10 hrs after surgery), hybridization was markedly increased in the hilus granule cell layers in the dentate gyrus and CA1 cell layers. GDNF mRNA levels peaked at 24 hrs after seizure onset and then declined to near control levels by 24 hrs. In these rats hybridization was not increased in other brain areas. RT-PCR analysis of total mRNA isolated from the dentate gyrus CA1 of hilus lesion rats demonstrated a 5-fold increase in GDNF mRNA compared to control GDNF555. Intraventricular injection of 0.5 ug kainic acid stimulated a similar bilateral increase in GDNF mRNA in the granule cells. However, at 10 hrs post-injection, hybridization to GDNF mRNA was also elevated in areas of neuronal damage in the isilateral hemisphere including stratum pyramidale of hippocampal region CA3, medial thalamus, and piriform cortex. These results indicate that the intense physiological activity of seizure is sufficient to induce GDNF mRNA expression in hippocampal granule cells but not in other populations of neurons engaged in the seizure episode (i.e. cortical pyramidal cells). The additional fields of hybridization in kainic acid-treated rats indicate that GDNF expression is also increased in some populations of neurons following neurotoxic insult. Supported by NS57658.

The role of brain-derived neurotrophic factor (BDNF) is distributed in many brain regions and regulated by excitatory neuronal activity. Despite numerous studies of BDNF mRNA, the production of BDNF protein is poorly understood because of a lack of sensitive and quantitative methods. Recently, we have established a two-site enzyme immunoassay (ELISA) for BDNF protein. The combination of high-affinity antibodies and functional detection minimized interference from other BDNF-related proteins. To investigate the hypothesis that BDNF mediates later changes in neuropeptide expression, we evaluated the temporal and spatial correlation between changes in BDNF protein and neuropeptide levels after limbic seizures. In hippocampal BDNF was rapidly increased about 2-fold by 4 hr, reached maximal levels at 16 hr, and remained elevated through 96 hr after seizure onset. In contrast, the BDNF increase was delayed in cortex and returned to the basal level by 24 hr. After an initial decline, neuropeptide Y content increased to control levels by 16 hr and was well above control levels at 24 hr. The peak neuropeptide content was also clearly increased by 16 hr after seizure onset in all areas. In contrast, hippocampal dopamine levels were markedly below control values from 2 through 96 hr after seizure onset. These data demonstrate increases in BDNF protein precede the major change in enkephalin and neuropeptide Y content, and thus could play an important role in neuropeptide regulation after limbic seizures.
540.5

Recent studies in our laboratory demonstrated that acute ECS increases the expression of BDNF (brain derived neurotrophic factor), and that chronic ECS enhances the induction of trkB protein. This work has been extended to study the regional regulation of BDNF and trkB, the protein tyrosine kinase receptor for BDNF, and to studies of CNTF (cAMP response element binding protein) in mediating the induction of BDNF by ECS. This study demonstrates that acute ECS (2 h) increases the expression of BDNF mRNA in several regions of hippocampus (dentate gyrus, CA1, CA3, and CA4), and the discrepancy between the adult and the neonatal expression may be due to a deficit in the remaining hippocampus. We hypothesized that hippocampal trophic factors may play a role in this deficit. We found previously that the projecting septum and locus coeruleus are dysfunctional after the neonatal hippocampal lesion. Moreover, previous work had suggested that trophic factors are down-regulated by trkB immunoreactivity in hippocampal neurons. Therefore, we have focused on the role of ECS in the regulation of BDNF and trkB. This study shows that ECS increased BDNF mRNA expression in hippocampus, which was significantly increased at 30% in the remaining hippocampus 4 weeks after a neonatal but not an adult unilateral lesion. Neither BDNF nor NGF mRNA was changed by ECS in the remaining hippocampus, which may contribute to the spatial memory deficit observed in a unilateral neonatal hippocampal lesion. Supp. NIH HD23315.

540.6
Regional Increase in Brain-Derived Neurotrophic Factor and Nerve Growth Factor mRNA, but not in Acute and Basic Fibroblast Growth Factor mRNA in kindling. K. Sato*, K. Kashihara, K. Morimoto**, O. Osumi, Y. Fujisawa, K. Aokiwa, T. Hayasaka and S. Kuroda. Department of Neurophysiology and Department of Neurology, Okayama University Medical School, Okayama 700, Japan; Clinical Research Institute, National Sanatorium Minamikawasaki Hospital, Okayama 701-03, Japan; Department of Neurophysiology, Kyorin University Medical School, Tokyo, Japan; and 761-07, Japan.
The levels of mRNA for nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), acidic and basic fibroblast growth factor (FGF) have been studied in the kindling model of epilepsy. Using in situ hybridization and 35S-labeled oligonucleotide probes, the induction of mRNAs was evaluated in the rat brain 1 h after generalized seizures induced by daily electrical stimulations from the amygdala. BDNF mRNA levels were increased in the granule cell layer of the dentate gyrus and the pyramidal cell layer of CA1, CA2, CA3 and CA4. The magnitude of increase in the dentate gyrus was 4- to 5-fold that of the sham-operated controls, whereas moderate increase was observed in other regions. The significant increases in mRNAs were observed in the granule cell layer of dentate gyrus, CA1, CA2, CA3, CA4 and amygdala. Acute FGF mRNA slightly increased in the granule cell layer of the dentate gyrus. No detectable changes, however, were observed in basic FGF mRNA in response to kindled seizures in the regions examined.

These results indicate that the mRNA induction of neurotrophic factors, especially BDNF mRNA expression in the dentate gyrus, corresponded to increases in metabolic and electrical activity associated with seizures or neuronal vulnerability consistent with the kindling model of epilepsy. The observed differences in the spatial pattern of the neurotrophic factor mRNAs suggest that the mechanisms underlying expression of individual neurotrophic factors in kindling are heterogeneous.

540.7

Unilateral neonatal hippocampal lesions cause a long-lasting impairment in spatial memory, whereas unilateral lesions made in the adult rat (3 mos.) were not followed by a deficit. The discrepancy between the adult and the neonatal lesions may be due to a deficit in the remaining hippocampus. We hypothesized that hippocampal trophic factors may play a role in this deficit. We found previously that the projecting septum and locus coeruleus are dysfunctional after the neonatal hippocampal lesion. Moreover, previous work had suggested that trophic factors are down-regulated in hippocampal neurons. Therefore, we have focused on the role of ECS in the regulation of BDNF and trkB. This study shows that ECS increased BDNF mRNA expression in hippocampus, which was significantly increased at 30% in the remaining hippocampus 4 weeks after a neonatal but not an adult unilateral lesion. Neither BDNF nor NGF mRNA was changed by ECS in the remaining hippocampus, which may contribute to the spatial memory deficit observed in a unilateral neonatal hippocampal lesion. Supp. NIH HD23315.

540.8
CONSTITUTIVE EXPRESSION OF BDNF AND TRKB mRNA IN RAT BRAIN WITH AGE AND LEARNING IMPAIRMENT. S.D. Croll*, N.Y. Jr., R.M. Lindsay, S. J. Wiegand, Regeneron Pharmaceuticals, Tarrytown, NY 10591.

mRNAs for the neurotrophin BDNF and its receptor, TrkB, have been localized in most regions of the adult rat brain. These messages peak reach expression late in development, and remain at high levels into adulthood. We examined the constitutive expression of mRNA for BDNF and TrkB in rat brain from young adulthood into old age. The levels of many neuronal mRNAs were increased in old age have been found to depend on the functional state of the animal. To determine if the constitutive expression of BDNF and TrkB mRNAs depend on the cortical status of aged, animals were preformed in the Morris water maze task, and were subsequently divided into aged learning-impaired and aged learning-unimpaired groups. Northern blot analyses were completed for olfactory bulb, neocortex, hippocampus, dienephalon, striatum, midbrain, hindbrain, and cerebellum of 4 mos., 13 mos, 24-26 mos (aged) learning-impaired, and aged learning-impaired rats. Results demonstrate that BDNF and TrkB mRNA levels during age in some, but not all, brain regions studied. Both BDNF and TrkB mRNA levels were increased in aged learning-impaired animals compared to aged learning-unimpaired animals. Northern analysis was performed for CNTF to test the specificity of these expression-related changes in mRNAs. CNTF mRNA did not show the same impairment-related changes as BDNF-mRNA. An in situ hybridization study of BDNF and TrkB mRNA expression with age and learning impairment is currently being conducted.

540.9
CHANGES IN NGF AND BDNF mRNA EXPRESSION INDUCED BY ENTORHINAL CORTEX LESIONS. M.L. Shipman* and D.G. Stern, Brain Research Laboratory, I.A.B., Rutgers University, Newark, NJ 07102.

Injury to the mammalian brain imparts plasticity processes (e.g. axonal sprouting) that may contribute to behavioral recovery of function after injury. Unilateral ablation of the entorhinal cortex/E in adult rats leads to robust sprouting responses by remaining afferents in the hippocampus (HC). Ablations from the contralateral HC and CA1 hippocampal projections sprout collateral terminations throughout the dentate and hilar neurons. Ablations from the septal nucleus also reinnervates portions of hippocampus. We were interested in examining expression of injury-induced neurotrophic factors and induction of axonal sprouting in the hippocampus caused by unilateral damage to the rat's E.

The effects of unilateral, electrolytic E lesions on NGF and BDNF mRNA expression were determined by non-radioactive Northern blot analyses. Seven days after lesion, the expression of NGF mRNA in the hippocampus ipsilateral to the injury, was increased by 20%, but did not change on the contralateral side compared to intact animals. An 40% increase in expression of NGF mRNA was also detected in the ipsilateral and contralateral HC at 15 days after lesion. The expression of BDNF mRNA in the hippocampus ipsilateral to the injury was elevated by 40% one week after E lesion by 15 days. The expression of BDNF mRNA on the contralateral side did not change at any of the time points in comparison to intact animals. These results indicate that NGF and BDNF may contribute to the induction or maintenance of sprouting in the hippocampus after E lesions. Supported by Ipsen Foundation.


**S40.11**

**DIFFERENTIAL EFFECTS OF THE ABSENCE OF RETINAL CONNECTIONS ON BDNF GENE EXPRESSION IN THE MOUSE VISUAL SYSTEM. M.H. Hanpin* and J.J. Reese, Department of Anatomy, Medical College of Ohio, Toledo, OH 43614-1645.

In the present study we examine how BDNF mRNA levels in visual target regions (superior colliculus, SC; visual cortex, vCtx) is affected by the absence of retinal projections. To determine the effect of retinal removal before innervation of the primary visual nucleus, we have taken advantage of congenitally blind ocular retardation (OR) mice in which the optic nerve was either completely or partially removed normal mice with bilateral eye removal either at birth (5d after initial retinocortical contact) or from adulthood. These protection assays indicate that BDNF RNA levels in the vCtx are normally about 30% greater than in the SC. While BDNF mRNA levels in the OR SC were comparable to those in normal mice, about 90024-1763 of BDNF RNA in both the SC and vCtx by 7d - an observation consistent with work showing a similar effect of dark-rearing and TNT on BDNF mRNA expression in vCtx of postnatal and adult rats (Castrì et al. *PNAS* 89:3444). Our observations suggest that different brain regions involved with visual input (1° & 2°) respond differently to eye removal during the perinatal period; BDNF gene expression was downregulated in the SC, while the vCtx showed upregulation. Continuing work will determine (1) how the normal postnatal increase in BDNF mRNA in the vCtx (which is attenuated by blocking light input to the brain) compares in the complete absence of retinal projection, and (2) the change in retinoreceptive layers of the SC. Supported by NIH Grant NS26777.

**S40.13**

**INDUCTION OF GLIA-DERIVED GROWTH FACTOR (S100B) IN RATS FOLLOWING CARDIAC ARREST. S.P. Jhas*, R.B. Matsusato and D.D. Truong, Dept. of Neurology, University of California Irvine, Irvine, CA 92717.

S100B, a small, dimeric, acidic calcium-binding protein, is known to stimulate neurite outgrowth and promote survival of serotonergic neurons. After resuscitation from 8 min of cardiac arrest (CA), Sprague-Dawley rats subjected to CA developed motor impairments, e.g. post-hypoxic asthenospermia. Biosynthesis and metabolism of 5-HT were also altered in post-CA rats; cortical 5-HT2A receptors were reduced; and treatment with the 5-HT2A agonist, (+)-1,2,5-dimethoxy-4-iodophenyl-2-amino-propanoic acid (DOI), significantly attenuated myelosis. These results indicated that function of serotonergic neurons may occur in post-hypoxic rats. In contrast, by 45 days following CA, motor functions of rats recovered and cortical 5-HT2A receptors of these animals were increased from low levels at early timepoints. In the present study, we, therefore, investigated involvement of S100B in the recovery process. Brain levels of S100B were examined with quantitative immunoblot analysis. Significantly increased levels of S100B were found in rats 14 days and >45 days post-CA (P < 0.05). The results indicate that reactive astrocytosis and elevated levels of S100B may participate in the recovery processes following hypoxic-ischemic insults to the brain.

**S40.14**

**NERVE GROWTH FACTOR (NGF) IS LOWER IN SEMEN FROM SPINAL CORD INJURED (SCI) MEN COMPARED TO NORMAL MEN. H. Fried, T. Ascher, M.D. Faust, J.L. Blackett, C.M. Lynne*, J.A. Weingartner and K.A. Crutcher, The Miami Project to Cure Paralysis and the Dept. of Urology, Univ. of Miami Sch Med, Miami, FL 33136; Dept. of Neurosurgery, Univ. of Cincinnati Med Ctr, Cincinnati, OH 45267.

In many cell types, NGF is required for normal cellular function in man. Sperm cells from SCI men are lower in motility and viability than sperm cells from normal men. The present study tested the hypothesis that levels of NGF in the semen of SCI men are lower than in the semen of normal men. SCI men were subjects in The Miami Project to Cure Paralysis. We analyzed the antegrad semen of 8 SCI men (mean age 39.3 ± 2.4 SEM, mean duration of injury 8.4 ± 2.5 years) and 9 healthy controls (mean age 31.2 ± 1.3 years). Complete semen analysis was performed on each sample. Semen was obtained in SCI men via electroejaculation (n=3), vibratory stimulation (n=4) or masturbation (n=1). Controls produced samples via masturbation. Whole semen samples were analyzed via a 2-site enzyme-linked immunosorbent assay (ELISA) for NGF. There was a ten-fold decrease in NGF levels in the semen of SCI men (0.03 ± 0.02 pg/ml) compared to normal semen (0.29 ± 0.07 ng/ml). This difference was statistically significant (p<0.01, ANOVA).

Our data indicate that the semen of SCI men has significantly lower levels of NGF than the semen of normal men. This condition may contribute to the asthenospermia and necrospermia seen in SCI men. Supported by The Miami Project to Cure Paralysis and by NIH grant NS31410.

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**NEUROTROPHIC FACTORS: EXPRESSION AND REGULATION VI**

**S41.1**

Expression of estrogen and androgen receptor mRNAs in immortalized oligodendrocyte cell lines. R. Stoika, L. Foster, A. Campagnoni, and P. Eispyve. The Laboratory of Neuroendocrinology, UCLA School of Medicine, Los Angeles CA 90024-1763.

Oligodendrocytes may mediate developmental effects of sex steroids on the CNS including the production of neurotrophins. The effect of the sex steroids, estrogen and androgen, in the development and regulation of oligodendrocytes has not been studied. To begin addressing this issue, we generated immortalized cultures of sex steroid-dependent oligodendrocytes. We examined proliferation and the expression of neurotrophins during development, we used a series of cell lines immortalized at specific stages in the oligodendrocyte lineage. (Stoika et al. *Dev Biol* 1995;15:100-109). All of the cell lines stained positively for markers of the oligodendrocyte lineage, A2B5, and 23'-cyclic nucleotide 3'-phosphodiesterase (CNP); none of the cell lines, however, expressed myelin basic protein which suggests that these were immature oligodendrocytes. Poly A+RNA was isolated from 10 oligodendrocyte cell lines. Northern blot analysis indicated that several of these lines expressed mRNA coding for the estrogen receptor (N7, N9, N11, N19) while mRNA for the androgen receptor was expressed in the N7, and the N19 cells. All of the cell lines expressed mRNAs coding for both brain derived neurotrophic factor and nerve growth factor. Cell lines identified to have specific steroid receptor mRNA's added to the N16 line, which does not express either estrogen or androgen receptor mRNA's, were grown at the permissive temperature (34°C) in a defined (serum and phenol red free) medium with and without sex steroids. These results suggest that sex steroids support the proliferation and differentiation of these cell lines and may also regulate the expression of neurotrophins that influence the development and survival of other cells in the nervous system. Supported by NS21220 and HD04612.

**S41.2**

β-INTERFERON INCREASES ASTROCYTE PRODUCTION OF NEUROTROPHIC FACTORS. P. Bonatsos and V. W. Yong*, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4.

Recent clinical evidence has suggested that β-interferon is efficacious in the treatment of multiple sclerosis although the mechanism of action remains unclear. Given that astrocytes can provide trophic support to neurons, and that the β-neurotrophic factors (CNTF) have been reported to protect oligodendrocytes against apoptotic damage, we examined whether β-interferon could increase the production of specific neurotrophic factors by astrocytes. Mouse β-interferon (1 to 1000 U/ml) was added to confluent or non-confluent neonatal mouse astrocyte cultures for varying periods of time. Total RNA was extracted and subjected to Northern blot analysis for levels of mRNA for CNTF, nerve growth factor (NGF) and the astrocyte filament protein, glial fibrillary acidic protein (GFAP). Doses of β-interferon from 100 U/ml significantly increased the mRNA for CNTF and NGF, but not for GFAP. Increase in mRNA for both CNTF and NGF (2-fold) was evident by 24 hours following β-interferon treatment. The β-interferon-enhanced trophic factor production was not reproduced by interleukin (IL)-2, IL-6 or γ-interferon. Western blot analysis is currently in progress at varying interferon levels. We conclude that the astrocyte production of neurotrophic factors by β-interferon may be relevant to its clinical efficacy.

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541.3 SYNERGISM OF PHORBOL ESTERS WITH IL-1β ON IL-6 RELEASE AND GENE EXPRESSION IN ASTROCYTES.
M. Grimaldi,* R. Arcone, A. Marino, G. Ciliberto and G. Schettini*  
Dip. Neurosciences, Se. Pharmacology, *CEINGE, Univ. di Napoli, Via S. Pansini 5, 80131, *IRCCS San Camillo-Forlanini, Rome, Italy

Cultured astrocytes produce and release interleukin 6 (IL-6) in basal and following stimulation with various agents. We have already demonstrated that Phorbol esters (PMA) cause release of IL-6. Moreover, it has been reported that secretion of IL-1β (1 ng/ml)-induced IL-6 by astrocytes PMA concentration-dependently stimulated IL-6 release with an EC50 of about 50 nM. The effect of PMA linearly increased from a maximal stimulation of secretion after 5 h. of exposure to a maximal stimulation at 24 h. At 24 h the concentration-related increase was calculated at 300 nM of PMA, indicating that PMA actions required at least 24 h to be fully explained. PMA effect upon IL-6 release by cortical astocytes was inhibited by the treatment with PKC blockers. Similarly, PKC-deactivation, by means of PMA pretreatment, caused a complete block of IL-6 release stimulated by PMA. The basal release of IL-6 resulted set at a higher level following PKC desensitization. IL-1β-stimulated IL-6 release concentration-dependently and its effect was potentiated by the blockade or by the desensitization of PKC. Conversely, calphostin C, over 24 h lasting incubation, did not affect IL-1β stimulation. Both IL-1β and PMA increased IL-6 mRNA expression, but IL-1β stimulated more consistently IL-6 mRNA expression than PMA. Finally, the co-stimulation of astrocytes with either IL-1β and PMA resulted in a synergistic stimulation of IL-6 release that was not observed by using PKC. This synergism was also found at level of mRNA expression. (Supported by MIUR 40%, CNR 9106012 and TP on Aging, AIRC 92-93)

541.4 BASIC FIBROBLAST GROWTH FACTOR (BFGF) EXPRESSION IN ASTROCYTES AND GLIOMA CELLS. M.K. Stachowiak, J. Moffett, K. Neary, J.R. Shapiro, W. Shapiro*, E.K. Stachowiak, Barrow Neurological Institute, Phoenix, AZ 85013

Expression of BFGF is transiently induced during a reversible transition of quiescent to proliferating reactive astrocytes. In constantly proliferating glioma tumor expression of BFGF is constitutively elevated. To model the molecular mechanics underlying induction of BFGF we developed cultures of human astrocytes and glioma cells. Confluent cultures of astrocytes were quiescent and did not express BFGF. By reducing cell density, events occurring in vivo in reactive astrocytes were mimicked: induction of BFGF proteins, their accumulation in the nucleus, cell hypertrophy and proliferation that lasted until a new confluent state was reached. Changes in the protein content reflected changes in the levels of BFGF mRNA. We used BFGF-luciferase reporter plasmids to show that the depletion of BFGF mRNA in confluent astrocytes results from repression by transcription and in mediates the changes upstream from the transcription start site of the BFGF gene. In U251 and SF776 glioma cells, BFGF proteins, mRNA, and BFGF-luciferase constructs were expressed at constitutively levels and were not susceptible to inhibition at confluent. The growth of the SF776 glioma cells was stimulated by either the 18k or the 22, 23, and 24 k/isoforms of BFGF expressed intracellularly from the CMV promoter, but not by enzymatically added BFGF. This suggests an intrinsic mechanism of action. The growth of astrocytes was stimulated by enzymatically BFGF. Our results suggest that: (1) BFGF participates in the regulation of the cell cycle in human astrocytes, and (2) deregulated expression of BFGF gene may underlie abnormal growth of glioma cells, and (3) deregulation of BFGF reflects altered trans-regulation of the BFGF gene promoter. Supported by APDA, AHA, and NIH.


Extracellular guanosine (Guo) and GTP affect both nerve cells (Gyngers and Pathman 3:997-1992) NeuroReport) and astrocytes (Kim et al. 1991) J. Neurosci. Res. 28:442. Guo or GTP also stimulate neurite outgrowth and branching from mouse hippocampal neurons co-cultured with astrocytes (J. Neurosci. Res. Abs. 1992). 19:24-7). Astrocytes in culture synthesize and secrete a number of neurotrophins, e.g. NGF, BDNF, FGF-2 and NT-3. We determined whether the neurotrophic effects of Guo and GTP might be due to stimulation of trophic factor production by the astrocytes. Guo or GTP (10 μM) increased the synthesis of FGF-2 and NGF mRNA by the astrocytes. Cultures treated with 24 hours total mRNA was extracted and analyzed by Northern blotting. Cultures treated with Guo or GTP increased both FGF-2 and NGF mRNA over control cultures. The astrocyte culture medium, as determined by ELISA, was also increased under these conditions. Since Guo and GTP stimulate neurite outgrowth from PC12 cells in the absence of astrocytes we questioned whether Guo and GTP stimulated the production of neurotrophins in PC12 cells. Both Guo and GTP induced c-fos as determined by Northern and Western blotting. This may lead to activation of other genes involved in neurogenesis. Moreover FGF-2 neutralizing antibody added to culture medium inhibited neurite outgrowth in PC12 cells. Guo. This raises the possibility that Guo induces FGF-2 synthesis in PC12 cells that contributes to their ability to enhance neurite outgrowth. Support: Hospital for Sick Children Foundation of Toronto and George M. Calder.

541.6 INCREASED 125I-IGF-2 BINDING IN THE COLCHECICINE LEDENED HIPSCampus: FUNCTIONAL mRNA EXPRESSION FOLLOWING NEURONAL CELL DEATH AND GLIOSIS. C.R. Bresser, M.M. Bresso, A. Costa, W.E. Sonntag, and S.S. Leonard. Department of Pharmacology Psychiatry, University of Colorado Health Sciences Center, Denver, CO 80262

IRISIL-like growth factors are a family of structurally related, weight peptide hormones which may act as neurotrophic factors in the central nervous system. We have identified NGF-1 and NGF-2 in the brain following cytotoxic colchicine lesions to the dentate gyrus of the hippocampus at times points up to 10 days post injury. We then examined the resultant increase at a time dependent increase in the-IGF-2 binding in the lesioned region and overlying cortex (Neuroscience Abstracts 269, 1991). Neuronal survival was studied by an antibody to glial fibrillary acidic protein, a marker of the granulol cell layer of the dentate gyrus, and extensive glial ingrowth into the lesioned and damaged areas. In situ hybridization revealed no observable mRNA for type-2 receptors in the lesioned area, while demonstrating normal expression to the ipsilateral CA1/CA3 and the contralateral hippocampus. Western ligand binding for IGF-2 binding proteins (IGF-BP) have suggested that the increase in IGF-2 binding may be due to expression of IGF-BP proteins. Therefore, we have examined for IGF-2 expression within the glial cell population. Our laboratory is currently examining colchicine lesioned animals for IGF-2 mRNA expression, as well as the expression of IGF-1, IGF-2, and other neurotrophic factors. Such expression would suggest that astrocytic IGF-BPs in response to neural injury, possibly to attract or maintain levels of the IGF peptides, which may in turn influence astrocytic proliferation and protein expression following neuronal cell death.


Previous studies from our laboratory and others have indicated that astrocytes produce neurotrophins. To understand regulatory mechanisms and the potential interaction of neuronal signals, we have evaluated effects of depolarizing signals (glutamate, kainate, and NMDA) on astrocyte NGF and BDNF mRNA. 

Purified astrocyte cultures were established from P1 rat brain forebrain using published techniques (McCarthy and DeVellis; O'Malley et al). The cultures contained > 95% astrocytes, revealed by GFAP immunocytochemistry. Solution 1 (6 mM KCl). Glutamate was assessed at a level of 0.025% of total RNA than BDNF mRNA (0.3 lg/mg of total RNA). The increase in both mRNA was determined at 24 h after exposure to field stimulation of neurotrophic expression, cultures were exposed continuously to KCl (25 mM) for 4 hours or 48 hours. Both 4 and 48 hours of exposure resulted in a significant increase in BDNF mRNA. Effects of the increase of NGF mRNA to 50% (48 hours). This increase in BDNF mRNA was relatively specific, since NGF mRNA was not similarly affected. Increases in neurotrophic expression at 48 hours were accompanied by significant decreases in GFAP expression and the alteration of astrocyte morphology from mature processes-bearing cells to more immature, ramified cells.

Our data suggest that depolarizing signals influence astrocyte function by modulating neurotrophin expression and astrocyte morphology. We are presently exploring the roles of neurotransmitters and mechanisms underlying these events (Supp: NICHHD HD23515 and the UMDNJ Foundation)
5-HT SELECTIVELY PROMOTES THE SURVIVAL OF O-2A PROGENITOR CELLS PREPARED FROM THE STRIATUM OF THE E14-RAT: INTERACTION WITH BFGF
J.W. Commissiong, T. Takashima, Jane M. Johnstone and R.I. Stanmeyer

We showed recently that oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells prepared from the striatum of the 516 rat, produce a potent neurotrophic factor that is secreted, and causes a marked increased survival of dopaminergic neurons in culture (Takashima et al., Neurosci. Letts. 166:187-192, 1994). A similar, target-derived factor that is transported retrogradely to dopaminergic neurons in the substantia nigra, might be produced in the striatum, in the adult, by an O-2A-like cell, in response to neurotransmitters released by dopaminergic neurons. Since dense dopaminergic and dopaminergic innervations are present in the striatum, we tested the effect of dopamine (DA), and 5-
hydroxytryptamine (5-HT), (and noradrenaline as a control) at 1 x 10^-8 M, for their ability to promote the survival of O-2A progenitors in culture, after plating at 3 x 10^4/cm^2. The cells were plated with serum (5.0%) for the first 24 hr, and then grown in a serum-free medium. DA was severely toxic. Only the small number of type-I astrocytes in the DA-treated cultures survived beyond the second day in culture (DIV). NE had no significant effect. 5-HT caused a significant protection (P<0.05). BFGF (10 ng/ml) caused a significant (P<0.05) inhibition of the toxic effect of DA. 5-HT + BFGF caused a significant (P<0.001) than that produced by BFGF alone at DIVS. Experiments to: determine if 5-HT is mitotic; 2) purify the putative dopaminergic neurotrophic factor (DNTF) produced by primary O-2A progenitor cells; 3) screen the striatum for additional positive cells during critical stages of development; 4) determine the dose-response effects of 5-HT and the 5-HT receptor(s) involved are in process. One function of the serotoninergic innervation of the striatum might be to provide neurotrophic support of an A2B5-like cell.

542.1
MODULATION OF GAP-43 mRNA EXPRESSION IN CULTURES OF RAT CEREBELLAR GRANULE CELLS

In situ hybridization studies from our lab have localized GAP-43 mRNA in granule cells of the developing and adult rat cerebellum (Rowe, Neurosci. Abst. 16:813, 1990). The level of GAP-43 mRNA expression was found to be developmentally regulated since higher levels of GAP-43 mRNA were present in the neonatal cerebellum as compared to adult cerebellum. The objective of this study was to determine the effects of putative cerebellar neurotransmitters (e.g. GABA and excitatory amino acids), acting at granule cell synapses, on GAP-43 mRNA expression levels.

Both GABA and glutamate have been implicated in granule cell trophic activity and their relative levels appear critical for granule cell maturation. The model systems chosen for our study were dissociated cerebellar cultures enriched for granule cells. Cells were exposed to agonists and antagonists of GABA and EAsAs in serum-free media for 7 days in tissue culture chamber slides.

 Cultures were hybridized with a 32P-labeled oligonucleotide to GAP-43 mRNA and the amount of hybridization within process-bearing cells was quantitated via an image analysis system. Results from these studies indicate that the addition of the inhibitory amino acid GABA (25 and 50uM), the GABAB agonist, baclofen (25 and 50uM), as well as the glutamate receptor antagonists, MK-801 (25 and 50uM) and CNQX (25uM), decreased GAP-43 mRNA expression. This study shows that GAP-43 mRNA expression in granule cell bodies could be modulated by synaptic input from both excitatory glutamatergic mossy fibers and inhibitory GABAergic Golgi interneurons. Thus, GAP-43 modulation by these neurotransmitters may contribute to the growth of parallel fibers and neuroplasticity at the parallel fiber-Purkinje cell synapse. (Supported by NIDA T32DA07237, NICED DC01094 and SEED from DuPnt)

542.3

It is currently believed that only isozyme BB of creatine kinase (BCK) initiates in all vertebrate neuromuscular junctions. In White Leghorn chicken and rat skeletal muscle, BCK is replaced by MB and finally by MM-CX. It is thought that BB-CX remains the sole type in brain. Here we found that Rhode Island chick brain and heart cytosolic (CCK) specific activities showed the lowest and relatively constant values from stage 30 up to month 8, when an abrupt increase occurred. CCK values from breast and thigh muscles increased 2235 and 4215, at hatching. At month 8, they returned to basal levels. On the contrary, CCK breast and thigh values increased 2100X and 3300X at year 1.5. Brain showed a transition pattern opposite to the accepted one. With creatine in heart, typical and atypical MB isozymes were present from stage 30 up to hatching; while varieties of MM and MB-CX were found in thigh and breast muscles. BCK-CX and varieties of MM and MB-CX were found from hatching up to month 8 in heart, thigh and breast muscles. Varieties of BB, MB and MM-CX isozymes were present in heart and thigh muscles, whereas no MB varieties were found in brain and breast muscle, coinciding with the most amniospecific period of ATP synthesis, when transphosphorylation is indispensable.

542.4
DIFFERENTIAL EXPRESSION OF GLYCINE RECEPTOR ISOFORMS BY EMBRYONIC RAT SENSORY CORD NEURONS DURING DEVELOPMENT IN VITRO. M. D. Wilber, and P.A. St. John, Program in Neuroscience, Dept. of Anatomy, University of Arizona, Tucson, AZ 85724.

It is well-established that acetylcholine receptors undergo a change in subunit composition during development at the neuromuscular junction. By comparison, relatively little is known about developmental changes in neurotransmitter receptors in the central nervous system. We are studying the developmental regulation of glycine receptors expressed by spinal cord neurons. Previous results show that embryonic spinal cord neurons undergo a change in strychnine-sensitivity of responses to glycine during the first week in culture. To rule out the possibility that strychnine-insensitive responses to glycine are a product of cross-activation of GABA receptors, we have used a monoclonal antibody, mAb 4A, raised against the alpha subunit of glycine receptor to provide independent evidence for the existence of glycine receptors expressed by these neurons. This antibody recognizes an epitope common to all glycine receptor alpha subunits cloned to date, but is not found in any of the glycine receptors cloned thus far. Initial results demonstrate that embryonic spinal cord neurons express glycine receptor alpha subunit immunoreactive material by the first day in culture. This is a time when these neurons exhibit strychnine-insensitive responses to glycine but before they bind strychnine in radio-ligand binding assays. This suggests that these neurons are expressing different isoforms of the glycine receptor at different times during development in vivo. (Supported by NIH and The Robert S. Flinn Foundation)
452.5 POSTNATAL DEVELOPMENT OF SERINE/THREONINE PROTEIN PHOSPHATASES: AN EXPEDITION INTO THE NEURODEVELOPMENTAL INTERACTION WITH MICROTUBULES. G.V.W. "Johnson and S.M. "Dudec". Dept. of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL 35224-0117.

Serine/threonine protein phosphatases regulate a wide variety of processes within neurons, and apparently play crucial roles in mediating appropriate structural changes during development. Such changes in the phosphorylation of specific structural proteins apparently modulate the organization of intracellular microtubules and can result in rearrangement of the cytoskeleton. The levels and activities of specific phosphatases and their association with microtubules during postnatal development were measured. Although the expression and activity of calcineurin (phosphatase 2B) was detected as early as postnatal day 1 (P1) in both the microtubule-enriched and whole homogenate fractions, it appears that calcineurin levels increase with age until P20, after which there is no further increase. These results differ from previous studies which showed no detectable calcineurin before postnatal day 4 (Pollit et al. 1991). Interestingly, calcineurin has been shown to exist in growth cones, and apparently depends on an intact cytoskeleton for this localization (Ferrara et al. 1993). In contrast to calcineurin, activity of phosphatase 2A is highest during the first postnatal week and declines thereafter. It is unknown at this time whether this increased activity early in postnatal life is due to increased levels of an isoform of the phosphatase 2A catalytic subunit, or from lower levels of the 2A regulatory subunits. Phosphatase 1 levels do not appear to change during this time. Studies are underway to determine the relationship between these changes in phosphatases which occur during postnatal development, and the age-related alterations in the phosphorylation state of specific microtubule-associated proteins.

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452.6 Oligonucleosides Antisense to Calmodulin mRNAs Inhibit Proliferation and NF-kB-Induced Genes. T.A. Zhang, G.A. Inohaya, and B. Weiss. Dept. of Pharmacol, Medical College of PA, Phila., PA 19129.

Calmodulin (CaM) is involved in numerous biological processes, including regulation of the cell cycle and cell differentiation. Using the PC12 phosphorylase cell line as a model for studying neuronal growth and differentiation, we have previously shown that nerve growth factor (NGF)-induced neurite outgrowth from PC12 cells was associated with increased levels of CaM and CaM mRNA. Oligonucleosides, chemical and oligo hybridization techniques have demonstrated that CaM and the CaM mRNAs are present not only in cell bodies but also in neurites of PC12 cells differentiated with NGF. In order to address the role of CaM in PC12 cell differentiation, we investigated the effects of antisense oligonucleosides and oligonucleoside-oligodeoxynucleotide (ODNs) targeted to the initiation sites of the CaM mRNA transcripts from the three CaM genes in PC12 cells. We first assessed the uptake of the ODNs into PC12 cells by incubating an FITC-labeled oligonucleoside antisense to the mRNAs from CaM gene I which was then incubated with the cells. Confocal microscopy revealed that the FITC-labeled ODN was taken up into the cytoplasm and nucleus. The addition of all three ODNs antisense to the mRNAs encoded by the three CaM genes produced a dose-dependent inhibition of the proliferation of the PC12 cells. The three CaM antisense ODNs also inhibited NGF-induced neurite outgrowth when present at concentrations of 16 μM each. Similar concentrations of an ODN having a randomized sequence did not significantly inhibit neurite outgrowth. Immunocytochemical analyses showed that the CaM antisense ODNs also reduced the levels of CaM in PC12 cells treated with NGF, supporting the view that the inhibitory effect of the CaM antisense ODNs on cell differentiation was due to their ability to block the expression of the CaM genes. Our results suggest that CaM is involved in the proliferation of PC12 cells as well as in their differentiation cells induced by NGF. (Supported by NS03724.)


It has been shown that protein kinase C (PKC) is translocated from cytosol to membrane fractions following memory formation in several species. CP20 is an ARF-related GTP-binding protein associated with memory, and is a high-affinity PKC substrate. We have investigated changes of phosphorylation during associative learning (Nelson et al. Science 1990). Both cp20 and PKC were identified in S. purpuratus oocytes by immuno-staining and fluorescent BODIPY-phalloidin assay, respectively. The identity of cp20 was confirmed by blot analysis of I PLC.

Treatment of oocytes with Brefeldin A, a fungal metabolite known to interfere with ARF-related proteins, resulted in a transient increase in free-intracellular Ca++ as assayed by Fura-2/AM imaging. Subcellular fractionation indicated that cp20 was primarily associated with free ribosomes and rough ER, while only small amounts were found in smooth ER, Golgi, or cytosol. Additionally, approximately 20% of the cp20 was also found in the nucleus. In smooth ER, approx. 30% of the cp20 was in the phosphorylated state, while in rough ER, >50% of the cp20 was found to be phosphorylated. Treatment of oocytes with BFA (200 nM) caused a marked reduction in cp20 recovered from ER, and resulted in a small increase in amount recovered from the nucleus. Previous results (Nelson et al., 1992) indicated that purified cp20 can markedly enhance the incorporation of P-32 uridine into mRNA. Thus, cp20 may play a role in conjunction with PKC in inducing the increases in mRNA and protein previously demonstrated with associative learning.

452.9 ONTOGENY OF [H]MK-801 BINDING TO THE NMDA-GATED ION CHANNEL. C.S. Li, J. He, J. Harasz*, R. Sivar, Laboratory for Developmental Neuroscience, Departments of Psychiatry and Neurology, Albert Einstein College of Medicine, Bronx, New York.

The NMDA receptor plays an important role in developmental plasticity. Phencyclidine (PCP) and PCP-like drugs (MK-801, ketamine) act as non-competitive antagonists at the N-methyl-D-aspartate (NMDA) receptor. We have earlier shown that PCP treatment led to permanent long-term changes in seizure susceptibility. Here we report the developmental profile of interactions of L-glutamate, glycine and spermidine with [H]MK-801 binding in the immature rat brain. Rat pups were sacrificed on specific days after birth. Forebrain crude synaptosomal membranes (CSMs) were isolated and prepared. Aliquots of CSM were incubated with [H]MK-801 in the absence or presence of L-glutamate, glycine and spermidine. Non-specific binding was determined in the presence of excess unlabelled MK-801. For autoradiography of brain sections from postnatal days 1, 5, 7, 10, 14, 18 and 21 were incubated with 10 nM [H]MK-801 in the absence and presence of excess nonradioabeled MK-801, washed, dried and exposed against tritium-sensitive film. [H]MK-801 binding in postnatal rat brain increases with age. Moreover, binding of [H]MK-801 binding to L-glutamate, glycine and spermidine were age-specific. These results may clarify the long-term neurobehavioral effects of postnatal exposure to noncompetitive antagonists.

Supported by: National Institute on Drug Abuse


The visual pathway of fish is an important model system to study molecular events that regulate and support optic nerve growth and regeneration. In addition, zebrafish embryos are amenable to direct observation and manipulation and are thus an excellent model to study molecular events that occur during embryogenesis. Our interest is in the role of intermediate filaments IFs in these processes. Although a precise physiologic function for IFs has not been determined, their distinctive cellular distribution and molecular diversity suggest that, in addition to a structural role, they may function to support cell differentiation and growth during embryogenesis. We have previously isolated and characterized several intermediate filament proteins from the goldfish visual pathway. Goldfish retinal ganglion cells express two novel neurofilament proteins designated plakelin and gefillin. In addition, goldfish optic nerve ganglia cells express keratin as their predominant intermediate filament protein. Currently we are characterizing the expression of these intermediate filament proteins in the zebrafish. NIH EY05212 to N.S.

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Calcium-calmodulin-dependent protein kinase (CaMK) plays an important role in the Crx* second messenger cascade and is capable of phosphorylating neurofilamentous proteins. We have used immunocytochemistry to examine the distribution of CaMK-II and the type IV intermediate filament protein (IFP) α-Internexin in the rat dorsal root ganglion (DRG) and spinal cord. Labeling for type IV IFP peripherin was used as a comparison, because it has been shown to preferentially label small dark DRG neurons. CaMK-like immunoreactivity (CaMK-LI) in the DRG was restricted to a subset of large-diameter DRG neurons. Virtually all cells positive for CaMK also showed heavy labelling for α-internexin, while the remaining neurons contained faint α-internexin-LI. However, a few small, brightly α-internexin-positive cells were not stained for CaMK. Both CaMK and α-internexin antisera labeled a subset of periphere-positive neurons. In the spinal cord, robust CaMK-LI was seen in the ventral motor horn and tract of Lissauer but not in the dorsal columns, with the strongest staining in lamina II, somewhat less in L I and faint labelling of III-IV. α-internexin labelled processes throughout the cord, but clearly showed afemms from the dorsal root entering both the dorsal columns and lamina I. Stained tissue was evident in lamina II. Strikingly, peripheral labelling of the dorsal horn was very similar to α-internexin, with extensive labelling of the dorsal columns, lamina I and III-IV, but little staining in lamina II. Despite the fact that both IFP antibodies labelled large cell bodies only faintly, both stained myelinated fibre bundles entering the dorsal horn medially and turning into lamina III, characteristic of Aβ low-threshold mechanoreceptors. Thus, it appears that the extent of DRG soma IFP-LI is not a good indicator of axonal IFP content.


Postnatal handling receptor (GR) gene expression and HPA function in the rat. The effect on GR expression appears to involve activation of intracellular cAMP levels. cAMP is known to increase the expression of a number of transcription factors, including activator protein-2 (AP-2) as well as NGF-A and NGF-B. In these studies we examined the acute effects of handling on the expression of mRNA for these factors in Day 7 pups that were handled and sacrificed 0, 30, 60, 90 or 120 minutes following handling. Levels of mRNA expression were determined using in situ hybridization on brain sections containing dorsal hippocampus.

Constitutive expression for all three transcripts was generally low, but detectable on Day 7 of life. Handling resulted in a modest, but reliable increase in AP-2 mRNA levels throughout the hippocampus. NGF-A mRNA levels were increased dramatically throughout the hippocampus. In both instances, the increase was observed for at least 120 min following handling. In contrast, handling reduced NGF-B mRNA levels to below detectable limits, and again the effect persisted for at least 120 min following handling.

The promoter region for the rat GR gene (Jacobson & Yamamoto, unpublished) contains putative binding regions for both AP-2 and NGF-A. Thus, handling could alter GR expression by increasing cAMP formation and increasing AP-2 and/or NGF-B expression. Interestingly, environmental enrichment, which results in increased GR expression in peripheral rats, also increases NGF-A and decreases NGF-B expression in rat hippocampus.


Homeobox genes are transcriptional regulators considered to be upstream components of the regulatory hierarchy of genes controlling development. Among these, the homeobox gene family is expressed in the forebrain during embryonic development. Since brain injury recapitulates early developmental events, we employed hypothalamic lesions that induce precocious puberty to begin examining homeobox brain genes may be involved in the early events that lead to the initiation of puberty. Using deoxyoxycytidinucleotides complementary to the antisense and cDNA that includes portions of the POU domain, and was isolated by RT-PCR, polymorphic domains from the anterior hypothalamus of immature rats subjected to puberty-inducing electrolytic lesions. Half of the almost 100 clones from a single experiment were found to contain POU domain sequences; only a few POU domain genes were previously discovered in the hypothalamus but now known to be expressed in brain. A predominance of Oct-2 sequences was evident as early as 24 hr after the lesion. Hybridization histochemistry revealed an increased prevalence of Oct-2 transcripts in reactive astrocytes surrounding the lesion site. Electrophoretic analysis of the DNA oktomer to which POUs proteins bind to initiate their actions demonstrated that hypothalamic nuclear extracts contain proteins with mobilities corresponding to at least two different Oct-2 proteins. One of these proteins formed a prominent protein-DNA complex with a portion of the 5′ flanking region of the triplet transacting growth factor alpha (TGα) gene harboring a presumptive POU binding site. Such a binding is implicated in the initiation of puberty and is also expressed in reactive astrocytes, the results suggest that the controlling the expression of this gene may be initiated via transregulation of TGα gene expression. Supported by NSF SGER Grant #IBN-9305583 and NIH grants HD18165 and RR0163.


The homeobox gene engrailed has been found in invertebrates and vertebrates, where it is thought to play a role in segmentation and neurogenesis. The function or even the presence of a vertebrate homologue in the nervous system is as yet unknown. We have used the MAB 4D9 (gift of Nipam Patel) to examine the expression of engrailed-like gene products in whole animals and in the CNS of developing mouse embryonic neuroblasts. Engrailed-like immunoreactivity (ELI) was found in the nucleus of a specific subset of cells in the CNS of both larval and adult Aplysia. In juveniles, there is only one ELI cell in the left cerebral ganglion (none on the right), a bilaterally symmetrical cell in the buccal ganglia, and a few scattered cells in the pedal ganglia. By contrast, the abdominal and especially the pleural ganglia contain several ELI cells. In the pleural ganglia, the left pleural giant (LP1) cell is immunopositive, and so are cells of the anterior, medial, and posterior clusters. In the pedal ganglia, in addition to a few immunopositive clusters of cells, the giant cell R2 is immunopositive, as well as cells that we have tentatively identified as R10-R12 and R13. That both R2 and LP1 are immunopositive is consistent with the idea that these cells are bilateral homologues arising from the same developmental program but merging with different ganglia (Hughes & Tau, 1993).

A cell count performed in the pleural ganglia indicates that there is a significant increase in immunopositive cells during juvenile development (p<0.01). Cells may be fated to express the engrailed-like protein before they migrate into the CNS, since preliminary evidence suggests that ELI cells are found in the body wall, a site for the patterning of the CNS.

Our results suggest that an engrailed-like protein is expressed in the nucleus of a specific subset of neurons in the CNS of Aplaysia, where its expression pattern is consistent with it having a role in determination of cell fate.


Serotonin (5-HT) is specifically accumulated by embryonic tissues in a manner analogous to the molecule's sequestration by the 5-HT transporter (SERT) at adult synapses. Notably, 5-HT transport antagonists block embryonic 5-HT accumulation and are used to craniofacial and heart abnormalities. These results suggest that transport per se may regulate a morphogenetic action of 5-HT in the embryo. A basic prerequisite to understanding the mechanism of this action is to describe the appearance and sequence of features that lead to morphogenetic changes. We have used immunohistochemistry in concert with in situ hybridization to document both SERT protein and gene expression in early mouse development. A fusion-protein antibody (CT-2) directed against the carboxy-terminal of SERT and SERT and protein probes related to serotonin have been used to analyze SERT gene and protein expression with fluoroxite- and serotonin-sensitive [H]5-HT uptake in whole embryo culture. Our results reveal a precocious and heterogeneous distribution of SERT mRNA in several sites throughout the embryo including craniofacial epithelia and mesenchyme, midbrain, lung, and heart. Correlation of morphogenetic events with the spatial and temporal expression will more clearly delineate the embryonic involvement of 5-HT in development and provide basic information necessary for interpretation of pharmacological and genetic disruptions of the transporter.
We have used non-radioactive in situ hybridization to localize choline acetyltransferase (ChAT) transcripts in the chick embryo. Previous studies have demonstrated that ChAT transcripts in neural crest derivatives in the periphery (Rowe et al., Development 111: 771). Here we report on their presence in two regions of the CNS: the hindbrain and the spinal cord. At the earliest time examined (stage 19) we find ChAT expression along the midline of the optic tectum, in an interventricular ventricular cell column in the hindbrain, and in scattered cells in the central core of the retina. By stage 25, when many neuron populations have differentiated in the hindbrain, we find expression in a ventricular wall of cells extending from the level of the trigeminal nerve to the isthmic region. The location of the ChAT-positive cells suggests that they are postmitotic, and that they may include but are not restricted to some of the central components of the trigeminal system. The expression patterns in the retina are similar to that observed earlier, but the ChAT-positive cells in the central retina are more numerous. Since the retina develops in a central to periphery gradient, we hypothesize that ChAT expression is highest in developmentally more advanced retinal cells.
MOLECULAR AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT

542.23

REGULATION OF NEURONAL GENE EXPRESSION BY TARGET INTERACTORS: Nova, L. B. Welschen* and M. Maragò. Dept. of Biomedical Sciences, Columbia University, New York, NY 10027

Developing neurons and their synaptic partners exchange information that can affect their subsequent expression of particular genes. For neurons that can innervate either of two very different targets, their resultant patterns of gene expression may be significantly different from each other. We are studying this interactive process in the Retzius (Rz) cell, an identified neuron that is found at a precise location in each segmental ganglion of the leech nerve cord and innervates one of two targets, either body wall or the sex organs. The Rz neurons that innervate the sex organs in body segments 5 and 6 have different morphology, physiology and function than those homologues in other body segments, but will attain the same characteristics if the sex organs are ablated early in embryogenesis.

Comparing the patterns of gene expression between Rz neurons with different targets should allow us to find the genes that account for the two Rz neuronal phenotypes. To this end, we have amplified mRNA from individual adult Rz neurons in sex and non-sex segments, made CDNA from these mRNAs, and then used a differential display technique to compare expression profiles. We have thus far identified in these profiles a population of genes that are expressed exclusively in either sex- or non-sex Rz cells, and are in the process of obtaining information about their sequences and developmental patterns of expression in the nervous system. This information will be used to understand how neuronal fate is determined by early interactions with potential synaptic partners.

542.25

Nova-2: A New Neuron-Specific RNA-Binding Protein Associated With Parvalbumin-Positive Neurons. Yolanda VL Yang, Ronald J Buckanovich, Robert B Durell*. Laboratory of Molecular Neuro-Oncology, Rockefeller University, New York, NY 10021 USA

The Nova proteins were originally identified as target antigens in the neurodegenerative disorder paraneoplastic opsoclonus-myoclonus-ataxia (POMA). Analysis of the cloned family member, Nova-1, revealed that it was a neuron-specific RNA-binding protein with developmentally regulated expression. Using antisense from a patient with POMA, we have now cloned a second Nova family member, Nova-2.

Nova-2 shares extensive amino acid sequence homology with Nova-1. Both have homology with the KH RNA-binding domains (KBDs) found in PMB-1 (the familial mental retardation gene), hNRNP-K, and the yeast splicing factor MER-1. Comparison of Nova-2 to Nova-1 reveals 100% amino acid identity in the three KBDs, indicating a possible shared RNA target. Between RBD2 and RBD3, Nova-2 contains a unique domain with several stretches of alaas and glycine and one proline-rich region, suggesting that functional differences between Nova family members may map to this region.

In vivo hybridization (ISH) and mRNA analysis indicate that Nova-2 expression is limited to neurons in discrete regions of the developing and adult mouse nervous system, but with a different pattern of expression than Nova-1. We are further defining the expression of Nova-2 by Northern, RT-PCR, and ISH to distinguish between the Nova family members. In summary, we expect that the Nova family of RNA-binding proteins regulates neuronal development and function through their action on RNA metabolism, by such means as alternative splicing, stability, or translational regulation.

542.26

ROLE OF AP-1 PROTEINS IN TRANSCRIPTIONAL CROSS-TALK AT SOMATOSTATIN CYCLIC AMP RESPONSE ELEMENT (CRE). C.L. Smytheck-Sexton* and D.E. Millburg. The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 and The University of Cincinnati, Cincinnati, OH 45267.

The mechanism by which specific gene regulation, such as developmental and tissue-specific regulation, occurs is largely unknown. Given the ubiquitous nature of transcription factors and the large number of genes containing regulatory DNA binding consensus elements, how subsets of genes are transcribed in particular cells at certain stages of development remains an open question. The finding that cross-talk occurs among the hsp70 protein families, the Fox/Jun family and the CREB family, at the TRE (12-O-tetradecanoylphorbol-13-acetate response element) and CRE provides a possible mechanism for the specificity of developmental- and tissue-specific gene regulatory events. Our finding that Jun is involved in complex of cerebellar and diencephalic nuclear protein extracts which bind to the CRE led us to speculate that Jun levels and/or transcriptional activity could mediate CRE-driven transcriptional responses, especially CRE responses which are temporopre spatially regulated. We tested this hypothesis with phorbol-12-myristate-13-acetate (PMA) and forskolin treatment of phaeochromocytoma (PC12) cells transfected with the CRE-driven promoter of the somatostatin gene, which is known to be developmentally and tissue-specifically regulated. PMA (50 nM) increased transcription of the somatostatin promoter, presumably through the CRE, as did 25 μM forskolin. Treatment with both agents resulted in synergistic stimulation of transcription. In addition, gel shift studies showed that treatment of PC12 cells with PMA and forskolin increased binding of nuclear proteins from these cells to the CRE. These data suggest that the ratio of transcription factors, such as Fox/Jun and CREB, which are capable of interaction at each other's cognate DNA sequence, is crucial to determining the specificity of transcriptional events.

CEREBRAL CORTEX AND LIMBIC SYSTEM II

543.1

CONNECTIVITY OF FETAL LIMBIC CORTEX TRANSPLANTED INTO NON-LIMBIC CORTEX AFTER LONG TERM SURVIVAL OF HOSTS. M.F. Barbe*. Physical Therapy Institute, Philadelphia, PA, 19140

Previous studies using a molecular marker of limbic phenotype as well as Dil tracing techniques, in combination with brain tissue transection, have examined the developmental specification of regional cortical differentiation. The present study was undertaken to determine if connection phenotypes observed in earlier short term survival studies were maintained after long term survival of hosts receiving grafts. Limbic perihinal (phr) or sensorimotor (Sm) fetal tissue from rats aged embryonic (E) 12-14 was transplanted into neonatal rat Sm cortex. Animals were sacrificed 65 days later and Dil crystals were inserted into the graft. After a 12-14 week incubation period, the location of Dil-labeled neurons and axons were mapped in cortex and thalamus. Homotopic Sm transplants had no limbic thalamic or contralateral cortical connections, and only 1 of 7 had a connection with limbic ipsilateral anterior cingulate cortex (ACC). Most E14 phr grafts in host region had hybrid connections: out of 12 total animals analyzed in this group, 11 had both limbic and sensorimotor connections when one considers contralateral and ipsilateral cortices as well as the thalamus, 9 had hybrid ipsilateral cortical connections, while 7 had hybrid thalamic connections (ventral posterior and lateral dorsal nucleus), the remaining having either just limbic or sensorimotor. Nova-2: A New Neuron-Specific RNA-Binding Protein Associated With Parvalbumin-Positive Neurons. Yolanda VL Yang, Ronald J Buckanovich, Robert B Durell*. Laboratory of Molecular Neuro-Oncology, Rockefeller University, New York, NY 10021 USA

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543.2


We previously reported that the number of GABA-immunoreactive (ir) neurons was increased in the anterior cingulate cortex (ACC), but not in the primary visual cortex (VC), in the rabbits prenatally exposed to cocaine (Wang and Murphy, Soc.Neurosci. Abst. 1993, 19:50). In the present study, we investigated the development of the subgroup of GABAergic neurons which are immunoreactive for the calcium binding protein parvalbumin. Since the major effect of cocaine is believed to be on the uptake of dopamine, we compared an area of cortex rich in dopaminergic input (ACC) with an area lacking dopaminergic input (VC) following prenatal cocaine exposure. Pregnant rabbits received intravenous injections of cocaine (4mg/kg, twice daily) from day six to day 29 of gestation. The volume of salicylate was injected systemically.

The brains of rabbit pups were examined at P10, P20 and P60. Immunocytochemical methods were used to visualize parvalbuminergic neurons. Quantitative analysis revealed a significant increase in the number of secondary and tertiary dendrites per neuron which were parvalbumin-ir in ACC in cocaine exposed brains in all age groups examined compared to controls. In VC, however, there is no difference between cocaine and saline animals in the number of secondary and tertiary dendrites per neuron which were parvalbumin-ir. In both ACC and VC, there were no significant difference between cocaine and saline in the number of primary dendrites per neuron which were parvalbumin-ir. E13 or E12 phr into Sm cortex grafts had any connections with limbic thalamic nuclei or limbic contralateral cortices, 2 were connected to ipsilateral ACC. The data suggest that projection patterns are subject to complex environmental influences. Supported by NSF grant 9209459.

Previous studies have shown that the entorhinal cortex (EC), a transient area of "pericalcortex," matures histologically and neurochemically earlier than neocortical areas. To determine if connections between the EC and hippocampus (HC) also develop in the neonatal period, an electrophysiological tracer dye (ChAT) was injected into the temporal horn of the lateral ventricle of newborn rats (PND 2-3) and projections to superficial and deep EC layers were identified. Injection into superficial parietal and occipital neocortex labeled radial glial fibers and Cajal-Retzius cells only. Injections of the optic nerve and tract labeled the lateral geniculate nucleus (LGN) specifically; evidence of eye-specific laminar segregation of projections to the LGN was seen with the optic nerve injections. The above findings demonstrate that cortico-cortical connections between EC and HC develop in the first half of human gestation, well before those in other areas, e.g. visual cortex.

To illustrate the potential of this method for studying HC/EC maturations, we injected dII into the medial entorhinal cortex (EC) of a 19-day CA thanatophoric dwarf (in which HC and HC develop abnormally). Labeling of radial glial fibers and a few Cajal-Retzius cells, appropriate for neocortex at that CA, was observed; EC-appropriate connections were absent.


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Postnatal (PN) development studies of GAD67 mRNA-containing neurons within the rat cerebral cortex have shown that the developing GABAergic system is more uniform than previously thought. Early hybridization studies have revealed a prominent population of GAD67 mRNA-containing neurons in the dentate gyrus at early ages (PN0-5NS). These neurons were highly concentrated both within and slightly above the outer border of the developing granule cell layer, creating a labeling pattern different from that of the adult. In order to determine whether the GAD67 mRNA-containing neurons observed on PN0-5NS are GABAergic, we examined PN5-10 GAD67 mRNA-containing neurons with immunohistochemistry for GAD67 and its catalytic subunit. In these early PN ages, GAD67 protein was evident in neurons occupying similar positions to the GAD67 mRNA-labeled cells, and the morphology of these neurons suggested that they were not granule cells. Birthdating studies of neurons labeled with bromodeoxyuridine at PN4, the birthdate of many GABA neurons in the dentate gyrus, revealed labeled neurons in positions corresponding closely to those of the GAD67 mRNA and protein-containing neurons at the early PN ages. This suggests that the neurons with early birthdates arrive in the granule cell layer at early ages and are GAD67-containing interneurons. Double labeling studies are being conducted to confirm this. These GAD-containing interneurons, due to their position and early appearance, could play a key role in the cytoarchitectural development of the dentate gyrus.

543.7 SURVIVAL AND TRANSMITTER SYNTHESIS IN RAT SEPTAL NEURONS FOLLOWING EXCITOTOXIC HIPPOCAMPAL LESION IN EARLY POSTNATAL DEVELOPMENT M. Mantsch, D.W. Kasper*, T. Neumann and M. Frotscher
Institute of Anatomy, University of Freiburg, P. O. Box 111, 79001 Freiburg, Germany

Target cells are thought to regulate the survival and transmitter expression of basal forebrain cholinergic neurons by supplying neurotrophic factors (Thoenen and Beaudet, 1988; Physiol. Rev. 68: 1264-1335). It has been shown in adult rats that septopallidal cholinergic neurons survived after excitotoxic ablation of their target region and retained their immunoreactivity for the terminal acetylcholine synthesizing enzyme, choline acetyltransferase (ChAT); see Sofroniew et al., 1990, Science 247: 338-342.

To investigate whether the hippocampus is essential for survival and transmitter expression of developing septopallidal cholinergic neurons, we have destroyed the hippocampus by unilateral injections of the excitotoxic amino acid NMDA in 3 and 10 day old rats. These lesions were made to bilateral septal regions that were immunostained using antibodies against ChAT or the calcium-binding protein parvalbumin (PV). The latter staining has been used to stain GABAergic neurons (Freund, 1989, Brain Res. 478: 375-381).

We have found that a large number of septal neurons retained their immunoreactivity for ChAT and PV, respectively, at both survival times despite the early loss of their target region.

We conclude from our experiments that the target region has only a limited influence on survival and transmitter expression of developing septopallidal neurons.

(Supported by the DFG: Leibniz Program and Fr 6204.-1).


DMAP45 and 55 were originally identified as 45 and 55 kDa proteins that co-sedimented with microtubules purified from Drosophila embryos (Srinivasan et al., J. Neurobiol., 24:15-32, 1993). DMAP45 is present in the cytoplasm of all cell-types throughout early embryonic development and also accumulates in the developing nervous system to levels above those found in other tissues. DMAP55 has a cytoplasmic distribution similar to tubulin and is associated with mitotic spindles. DMAP45 has also been shown to be present in several nonneural cells in culture. In undifferentiated N1E-115 neuronaloma cells, DMAP45 is present early in the actin-rich region of the lamellipodia and microtubules protruding from the cell membrane, but is absent from the cytoplasm. Upon differentiation with DMSO, the protein is enriched in the distal tips of advancing growth cones and in microtubules protruding from the neurite shaft. In the fibroblast NIH-3T3 cells line, DMAP55 stains the endoplasmic reticulum.

DMAP55 is present along the lengths of the N1E neurite shafts in differentiated cells, and co-localizes with cytoplasmic microtubules in both undifferentiated and differentiated NIH-3T3 cells.

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453.9

A set of novel microtubule-associated proteins (termed DMAPs) (previously termed FLAT-MAPls) have been identified in Drosophilia and are present in the purified microtubule protein fraction of the rat brain. Developmental regulation of DMAPs has been observed in the rat cerebral cortex and cerebellum (Comery et al., Soc. Neu. Abst. 1993). The late developing nature of the dentate gyrus and the stereotyped pattern of connectivity within the hippocampal formation provide an ideal system to investigate the potential involvement of these proteins in process extension, differentiation and synaptic formation. Expression of DMAP45R in somata and dendrites of the pyramidal cells of the CA regions and the granule cells of the dentate gyrus correlates with process extension and cell differentiation. Expression of DMAP45R in the CA3 pyramidal cells correlates with the innervation of these cells by mossy fibers from the dentate gyrus. DMAP45R is also expressed in the inhibitory interneurons of the hilus and CA3 region with peak expression occurring at P9. DMAP55 immunoreactivity in axons correlates with the development of the mossy fiber projection from the granule cells of the dentate gyrus to CA3. The level of expression of DMAP55R increases during the growth of the mossy fiber system, reaching a peak level at P20, following which the level of expression gradually declines. Similar patterns of immunoreactivity during the development of other fiber systems suggests a possible role for DMAP55R in axonal extension. Supported by NIMH 53231 and ROI GM47962-01.

453.11

The possible role of cytoskeletal elements in experience-dependent plasticity was investigated. The antibody DMAP45 raised against a novel microtubule-associated protein isolated from Drosophilia cross reacts with a developmentally regulated antigen (DMAP45R) in rat brain (Comery et al., Soc. Neu. Abst. 1993). We examined the immunoreactivity of DMAP45R in layer 5 in the visual cortex of monocularly deprived rats. One eye of rat pups was sutured closed at P12. A nonremovable control group was used for comparisons. Coronal sections of the visual cortex were stained with DMAP45. Pyramidal cells were classified on the basis of immunoprecipitation in the soma and associated dendrites (soma/dendrite-positive cell) or in the soma only (soma-positive cell). The number of these two types of cells per unit area (NA) was determined in the visual cortical regions OIbm (somato-bisomatocellular) and OICb (bisomato-bisomato) contralateral to the deprived eye.

Deprivation until day 90 resulted in a reduction of DMAP45 somata/dendrite-positive cells in OICb. Removing the sutures at day 75, and allowing 5 days of exposure to light, restored the staining to control levels in the soma/dendrite-positive cells in OICb. The density of soma-positive cells was not changed in OICb in either condition. No differences were observed in the densities of either soma/dendrite-positive or soma-positive cells in the bisomato cellular region. The modulation of DMAP45R by visual experience suggests that MAPs may play a role in experience-dependent structural plasticity. Supported by NIMH 39603, NSF BNS88 21219, GM47962-01.

453.13
BRAIN MICRO-EEG FREQUENCY OSCILLATIONS. C.C. Turben®
Coghoun University School of Medicine, Division of Anatomy, Omaha, NE 68178

In these studies recordings macro and micro electrode recordings from the cat brain, micro-EEG, are considered. Combinations of ion currents give rise to complex patterns of neuronal electrical activity in the brain cell microenvironment, the extracellular space. The flow of ions through populations of ion channels in the neuronal plasma membrane and give rise to transmembrane ion currents. It is the sum of various currents flowing at any point in time that determines the neuronal membrane potential. The multiple ion channels with their diverse and interacting regulatory mechanisms allow the neuron to modulate its electrical properties in complex ways of high frequency oscillations and electrical fields.

Some of the cortical and hippocampal neurons have ionic conductances organized to endow them with auto rhythmicity. In many neurons the kinetics of these ionic voltage dependent conductances are such that the cell may respond preferentially to inputs at a certain frequency or frequencies acting as a resonator. There are interactive processes between the components of the dorsal hippocampus micro-EEG and the evoked potentials off the cortical-EEG and the micro-EEG.

These findings reflect the interaction between the dorsal hippocampus and cerebral cortical regions in the processes of ordering, sequencing the succession patterned sensory information as it relates to memory and conscious states.
544.1 POSTNATAL NEUROGENESIS IN THE DEVELOPING RETINA OF THE BRAZILIAN OPUSCULAR, O. STEWART, C. JENSEN, J. ISLAM, L. ELMQUIST, C. D. JACOBSSON2 and D. S. SAKAGUCHI. 1, 2Dept. of Zool. and Genetics, Signal Transduction Training Group, 1Neuroscience Program and 2Dept. of Veterinary Anatomy, Iowa State University, Ames, IA 50011, 2Department of Neurology, Harvard Medical School, Boston, MA 02115.

We are investigating cellular genes in the retina of the Brazilian opuscular, Monodelphis domestica. Monodelphis expresses both rod and cone photoreceptors and has two populations of ganglion cells: afferent and efferent. Using autoradiography and single cell labeling with [3H]thymidine, we demonstrate the presence of four cell types: afferent, efferent, a few undifferentiated cells, and a population of cells that are not labeled by [3H]thymidine. The undifferentiated cells express a variety of markers, including the photoreceptor markers VIP and TrkA, the afferent markers SNAP-25 and p65, and the efferent markers SNARE and Rab3. These results suggest that Monodelphis has a unique pattern of retinal development, with a large number of undifferentiated cells that may be involved in the regulation of retinal development.

544.2 SPATIAL AND TEMPORAL DISTRIBUTION OF SYNAPE-SPASE ASSOCIATED PROTEINS IN THE DEVELOPING RETINA OF MONODELPHIS DOMESTICA. D.S. Sakaguchi1, 2, J.J. Swanson1, 2, and C.D. Jacobsson1, 2, 1Dept. of Zool. and Genetics, Signal Trans. Training Group, 2Neurosci. Program, and 2Dept. of Vet. Anatomy, Iowa State Univ., Ames, IA 50011.

In the present study, we have examined the early postnatal development of the retina in the Brazilian opuscular, Monodelphis domestica. Monodelphis is a small, pinniped marsupial that has a unique pattern of retinal development. The retina is composed of two populations of ganglion cells: afferent and efferent. Using immunohistochemical methods, we demonstrate the presence of four cell types: afferent, efferent, a few undifferentiated cells, and a population of cells that are not labeled by [3H]thymidine. The undifferentiated cells express a variety of markers, including the photoreceptor markers VIP and TrkA, the afferent markers SNAP-25 and p65, and the efferent markers SNARE and Rab3. These results suggest that Monodelphis has a unique pattern of retinal development, with a large number of undifferentiated cells that may be involved in the regulation of retinal development.

544.3 DETERMINANTS OF RETINAL SPECIALIZATION: A NEW APPROACH USING FINITE ELEMENT ANALYSIS. Allen Diner, Department of Cell Biology and Anatomy, New York Medical College, Valhalla, New York 10595.

Neither differential cell death nor differential retinal ganglion cell histogenesis adequately accounts for the progressive increase in the central/peripheral (C/P) retinal ganglion cell (RGC) density ratio during the course of ontogenesis in the cat. Differential retinal stretch remains the only mechanism that could account for the final RGC topography. However, this hypothesis cannot be tested directly. This model assumes that the central retina stretches less than the peripheral retina. Differential stretch of the RGC layer could occur if central retina were thicker than peripheral retina. Retinal thickness was determined in E35 cat retina indicate that the RGC layer of central retina is 2.5 times thicker than that of peripheral retina. The thick spot is located about 0.8 mm from the optic disc. This thickness difference is unrelated to differences in the size or density of RGCs in central vs. peripheral retina. By E62 the C/P ratio becomes 18.3:1 and the distance between the optic disc and the area centralis is about 2.5 mm (Liu et al., '87). The shape of the optic disc to the retinal margin, was digitized. The RGC layer was modeled as having uniform material properties. A finite element analysis model was used to stretch the E35 RGC layer from 2 to 10mm in length. The model allowed making measurements of RGC density, as well as distance of the area centralis from the optic disc, over the entire duration of the stretching process. In a retina stretched from 2 mm to 8 mm in length, the obtained C/P ratio was 19.3 and the optic disc area centralis distance was 2.5 mm. These values are very similar to the in vivo results reported by Liu et al. (87). Therefore, the adult RGC layer topography can be predicted accurately by the non-uniform geometry of the RGC layer and by retinal stretch.

544.4 INSULIN-LIKE GROWTH FACTOR 4 (IGF-4) STIMULATES PROLIFERATION AND NEURONAL PROCESSES IN THE ADULT GOLDFISH RETINA IN VITRO. S.E.M. Bouchet* and P.F. Hitchcock, The University of Michigan, Dept. of Ophthalmology, Anatomy and Cell Biology, and Neuroscience Program, Ann Arbor, MI.

The retina of the goldfish grows throughout the life of the animal and regenerates when injured. We developed an organ culture system to test peptide growth factors for their ability to stimulate the proliferation of neuroretinal precursors in both normal and injured/regenerating retinas. Normal eyes were enucleated and the anterior structures removed. Eyecups were incubated for three days in Media 199, with or without a peptide growth factor. Cultures were posteriorly sliced with a bromodoxystilurea (BUD) before incubation was halted. Eyecups were fixed, frozen, sectioned and processed for BUD immunocytochemistry. The number of BUD labeled photoreceptor neuronal precursors (in the circumferential marginal zone) and rod precursors (in the outer nuclear layer) were counted and averaged. For retinas incubated in media alone, the average number of BUD-labeled neuronal precursors ranged from 5 to 15, and BUD-labeled rod precursors ranged from 1 to 8. For retinas cultured with IGF-I at concentrations ranging from 10-4 to 10-6 M, the average number of BUD-labeled neuronal precursors was 56, 113, and 154, respectively. In contrast, there was no significant increase in the number of BUD-labeled rod precursors. In summary, we show that IGF-I at concentrations of 10-4 to 10-6 M causes a 10-20 fold increase in the number of BUD-labeled neuronal precursors, but no effect on the number of rod precursors. Furthermore, we find that IGF-I is likely to be synthesized in the retina, and that IGF-I may play a role in regulating cell proliferation in both the developing and regenerating retina. Supported by NIH (NSI) grants EY07060 and EY07003 (CORE).
544.7 A START AT MAPPING GENES THAT CONTROL RETINAL GANGLION CELL NUMBER: ANALYSIS OF INBRED, F1 HYBRID, AND RECOMBINOINT INBRED STRAINS OF MICE. R. W. Williams*, D. S. Rice, and D. Goldowitz. Department of Anatomy and Neurobiology, University of Tennessee, Memphis, TN 38163

Genes that control neuron number play a critical role in the function and evolution of the vertebrate CNS. We have identified one, or possibly two gene loci that are responsible for normal strain variation in the number of ganglion cells in the mouse retina.

Ganglion cell axons were counted on electron micrographs of optic nerves taken from more than 140 animals belonging to 33 different inbred strains of mice, including strains 129, A, AKR, BALB/c, CH/Hs, C57BL/6, C57BL/Ks, CAST/Ei, CBA, CD-1, CE, DBA/2, LF, NZB, NZW, PL, SL, and STREP/El. Cell number varied substantially among strains, from a low of 49,000 in CAST/Ei to a high of 67,000 in NZW. Errors of the mean are typically 2,000. Strain averages are distributed bimodally, and most strains have populations that cluster close to 52,000 or 64,000. There is no correlation between cell number and body size, brain weight, or retinal area. Phenotypes of F1 offspring from crosses between high and low strains (BALB/c by C57BL/6, BALB/c by A, and PL by SJL) are consistent with a model involving two gene loci. However, we cannot yet formally rule out a model involving a single locus with three or more alleles. Ongoing analysis of recombinant inbred BXD lines of mice suggests that one of these loci controlling neuron number in the mouse retina maps to chromosome 1, 2, or 6.

Supported by NEI R01-8868.

544.9 ASYMMETRIC RETINAL GROWTH IN ADULT GREEN SUNFISH D.A. Cameron, L. J. Butler, and M. F. Gather. Dept. Biology, University of Michigan, Ann Arbor, MI 48109-1048.

Teleost fish retinas grow throughout life by a ballon-like expansion and by the appositional addition of neurons and glia to the retinal margin. An earlier report (Cameron & Easter, Vis. Neurosci. 10:375, 1993) presented the hypothesis that fish retinas with a curved, non-fused embryonic fissure grow asymmetrically along the retinal margin. We have tested this hypothesis in adult green sunfish (Lepomis cyanellus), which have a curved, non-fused embryonic fissure, concave towards temporal retina.

The thymidine analogue BrDU was intraocularly injected into adult fish (n = 6) and whole-mounted processed for fluorescence anti-BrDU immunohistochemistry 20-37 days later. In retinas an annulus of BrDU-positive nuclei was observed central to the retinal margin (but not along the embryonic fissure). The absolute rates of retinal growth were roughly exponential functions of fish size—small retinas tended to grow faster than large retinas. However, growth asymmetry was present: the normalized distances of the BrDU annulus to the nearest retinal margin were 1.00 ± 0.03, 1.27 ± 0.03, and 1.44 ± 0.09, in temporal, dorsal, nasal, and ventral retina, respectively (means ± SEM). Thus temporal and dorsal quadrants enlarged less than ventral and nasal quadrants. The normalized densities of cone photoreceptors in the same quadrants roughly matched the normalized growth distances (1.00, 1.03 ± 0.09, 1.19 ± 0.05, 1.55 ± 0.14). Growth asymmetry was thus attributable to differential cellular addition rather than differential passive expansion.

Supported: NIH grants EY-00168 and EY-00469, and a Sokol award.

544.10 NEUROPEPTIDE RETINILNE AS A PROTECTOR OF RETINAL DYSTROPHY IN CAMPBELLS RATS. N. Balasubramanyam*, N. Balasubramanyam, A. Ragno, and W. A. Cameron. Institute of Neurological Sciences, University of Edinburgh, Edinburgh, Scotland.

Neuropeptide retinalilne (NPR) is a 39 amino acid peptide that inhibits the release of dopamine (DA) from dopamine (D1) receptors in the retina. It is also a neuroprotective agent that has been shown to reduce the loss of dopaminergic neurons in the retina of adult rats. The mechanism of protection is thought to be mediated through a reduction in the release of DA from dopaminergic neurons in the retina.

The retina of adult rats was isolated and exposed to the dopamine D1 receptor agonist quinpirole. Pharmacologically, this induction of dopamine release was reduced by pretreatment with NPR. This was suggested by a significant reduction in the dopamine D1 receptor agonist-induced rise in cytosolic calcium. This result indicates that NPR serves as a natural protective agent against dopamine-induced oxidative stress.

The authors propose that NPR could be a potential therapeutic agent for the treatment of retinitis pigmentosa and other retinal diseases. They suggest that further study is needed to determine the exact mechanism of action of NPR and to explore its potential as a therapeutic agent.


Neuronal expression of the early oncongenes c-fos and c-jun has been reported following stimulation of different regions. 30/35-day-old Wistar rats were chronically illuminated with 1200 lux during 8 days, followed by different dark adaptation periods. Illumination produces degeneration of photoreceptor outer segments. It is known that this phenomenon is reversible after an adequate period of darkness. Retinas were fixed by perfusion with a 4% paraformaldehyde solution, in phosphate buffer, after a period of illumination followed by 1, 3, 6, 10 and 18 days of dark period. Cryostat sections were immunocytochemically stained with antibodies to c-fos and c-jun (Affinity Bio-research Prod. A1000) and c-jun (CRB) c-dil (1:10000), biotinilated donkey anti-sheep IgG (Sigma) and the extravidin-peroxidase complex (Sigma). C-fos and c-jun immunoreactivity was detected in the somata of photoreceptors in all retinas thus observed. It was maximum in the photoreceptor nuclei of rats kept in total darkness for 3 days, and decreased thereafter. Staining stabilization was reached at about 10 days post illumination. The conclusion is that these expression changes played a role in the molecular events that participate in photoreceptor regeneration.

544.12 EXPRESSION OF D1 AND D2 Dopamine receptors and dopamine transporter mRNA in the adult and fetal monkey retina. A.K. Ronkainen, L. Chai, and W.S. Chai. Oregon Regional Primate Research Center, Beaverton, OR 97006 and Dept. of Physiology, OHSU, Portland, OR 97201-3098.

Expression of D1 and D2 dopamine receptors and dopamine transporter (DAT) mRNAs were studied in the adult and fetal monkey retina by Ribonuclease Protection Assay (RPA). Eyes were dissected from adult and a 70 day old monkey fetus. Monkey specific cDNA clones corresponding to the carboxy terminus of the rhesus monkey D1 and D2 receptors and to the XI-XII transmembrane domains of monkey DAT were cloned and used to generate the cRNA probes. The standard curves were constructed using known amounts of synthesized sense RNA. In the adult monkey retina, dopamine D1 and D2 receptor mRNAs were expressed in equal quantities and at relative high levels. DAT mRNA was also expressed in the retina, but at a more moderate level. In the 70 day fetal eye, D1 and D2 receptor mRNAs and DAT mRNA were not detectable. The dopamine D1 receptor mRNA was higher than D2 receptor mRNA and D2 receptor mRNA higher than DAT receptor mRNA. Thus, several of the genes of dopaminergic neurotransmission are expressed early in the developing fetal eye of rhesus monkey. (Supported by DA07165)

We have previously demonstrated that extracellular levels of endogenous glutamate are high in the developing rabbit retina. This amount of glutamate would be toxic in the adult retina; however, immature neurons survive and maintain normal developmental processes. We wished to determine whether NO which has been postulated to mediate glutamate toxicity in the adult CNS may be present and function in the developing and adult retina. First we used immunocytochemistry and histchemistry to localize nitricergic neurons in the adult retina and subsequently determined developmental profiles. Secondly using retinal explants we have analyzed viability of immature and adult neurons in response to NMMA and NO antagonist and agonist. Results show that prototypic nitricergic neurons are mature early but do not express detectable levels of NOS until day 10. Furthermore immature neurons at day 1 are insensitive to glutamate toxicity, while in the adult retina dose-sensitive toxicity is induced by NMMA and inhibited more than twofold by methyl arginine. We conclude that 1) glutamate toxicity in adult retina may be regulated by NO and 2) the resistance of immature neurons to glutamate toxicity may result from a delay in the maturation of the NO system. Supported by EV01655, EY06068 and Fight for Sight.

544.1

ELECTRIC EFFECTS ON AXONAL TRANSPORT IN THE CORTICOSPINAL TRACT FOLLOWING SPINAL CORD INJURY. J.A. McLear*, T. Cobb and T. Khan, Rehabilitation Research & Development Center, Edwardw C. McC. VA Hospital, Havana, IL 61245.

Unlike axons of peripheral nerves, injured axons of the adult spinal cord seldom undergo a functional regeneration. Recent studies have shown that a direct current field at the injury site enhanced regenerative outgrowth, and stimulates directed neurite growth. Spinal axonal injury in adult rats temporarily increased the level of slow axonal transport using tritiated amino acids injected in the retina. The present study was designed to test the hypothesis that electrical stimulation increases neurite regrowth by enhancing this period of increased axonal transport following spinal cord injury. Adult rats received a dorsal spinal cord hemisection at the T8 level. Miniature stimulators were implanted at the time of surgery with platinum disk electrodes placed extradurally proximal (anode) and distal (cathode) to the injury. The stimulators provided a constant electrical current to the conduction tract was labelled with 3H-methionine one week, five weeks, or fourteen weeks later by injecting 200 uL of radioactive vehicle into the sensorimotor cortex. One week (fast transport) or three weeks (slow transport) after the isotope injection the animals were perfused with formalin, the entire spinal cord dissected, and the radioactivity in 5 mm segments of the spinal cord determined by scintillation counting.

We found that the amount of radionuclide delivered by slow axonal transport was consistently (but not statistically) higher in the spinal cords of animals treated with electric fields. The spinal cord segment containing the injury site in the electric field treated animals did contain statistically more (15%) label relative to sham animals (no field) one week after injury. This increased label at the injury site disappeared after five or fourteen weeks. Fast transport was not altered at any time point, and therefore likely did not contribute to the increase. These data suggest that electric fields increase the slow transport of materials within the conduction tract, and temporarily prevent axon die back and/or stimulate neuronal sprouting near the injury site.

Supported by the Rehab. R&D Service of the Department of Veterans Affairs.

544.2

LONG-TERM EFFECTS ON RETINA OF RHESUS MONKEYS FED TAURINE-FREE HUMAN INFANT FORMULA. H. Imaki*, M.D. Neuringer and J.A. Sturman, NY State Institute for Basic Research in Developmental Disabilities, Staten Island, NY; Oregon Regional Primate Research Center, Beaverton, OR.

We have been studying the effects of postnatal deprivation of tauring by feeding rhesus monkeys a infant formula alone or supplemented with tauring from birth. Previous reports have documented reduced tissue tauring concentration and smaller ileal bile acids concentrations with tauring in tauring-deprived monkeys at 3 months and 6 months, although at 12 months these parameters were no longer different, suggesting the biochemical dependence of intestinal tauring synthesis has at least by 6 months, but has regressed by 12 months. At the early ages, the tauring-deprived monkeys have deficits in visual acuity and morphological changes in the retina and visual cortex compared with monkeys fed the same diet supplemented with tauring. The retinal changes persisted at 6 and 12 months. The present study addresses the question of whether or not the retinal changes persist for a substantial period beyond the time at which there are no longer reduced tissue tauring concentrations. Rhesus monkeys raised for 4 years were examined, and although there were no differences in tissue tauring concentrations between tauring-deprived and tauring-supplemented monkeys, as might be expected from our previous results, all tauring-deprived monkeys showed retinal changes, indicating that the retinopathy of tauring deficiency persists long after the differences in tissue tauring concentrations have disappeared. Supported by NH grants HD-18678 and RR-00163.

544.3


Although it has been shown that electrical stimulation can accelerate sprouting, it is not clear whether increased neuromuscular activity during sprouting has any effect on the size of motor units (MU) once connections are made. In this study, this question was addressed using high levels of activity during sprouting and determining the size of enlarged MUs in partially denervated muscles (PD) once connections were re-established. MUs were divided into 2 groups, exercised (E) and sedentary (S) in which rats received large cage running wheels (E) or in small cages with limited movement (S). After 4 weeks of exercise training, rats were killed, and hindlimb muscles were partially denervated by cutting either L5 or L4. Four weeks later, MUs were quantified in the plantaris (PL) and gastrocnemius (MG) and tibialis anterior (TA) muscles were prepared for muscle and EMG recording in PD and controlateral control (CC) large and small. Size and size of all of 4 muscles, the size of the enlarged MUs in PD muscles was significantly larger than in CC. In PD, the MU number was different in E and S. For an average of 50% PD, 4000 MUs were counted (E) vs 15.6 ± 0.82 (S) as compared to 10.71 ± 0.9 in CC muscles. For MG, the size of MUs of 6.8 ± 1.91. 8 DEV 9.6 (S) and 15.6 ± 0.82 (S) 10.71 ± 0.9 CC. Since these muscles were significantly higher in the number of MUs, the MG force was increased by the number of muscle fibers per motor unit and therefore of sprouting. These results show that high levels of neuromuscular activity do not influence the capacity of remaining motoneurons to form enlarged MUs. Supported by MHIC.

Several previous studies have shown that when the rodent spinal cord is damaged, DC electrical field treatment (DCEF) can result in partial recovery of function. The objectives of this study were to investigate the conditions which determine whether such recovery involves regeneration or sparing of axons. The methods incorporated a spinal hemisection in rats followed by DCEF (Traxon®) for 6 weeks, and then sacrifice. Control animals received no DCEF. Prior to sacrifice, animals were injected with WGA-HRP isplateral and caudal to the lesion. After histological processing, WGA-HRP transport to the red nucleus was determined by automated grain counting. The results showed that all DCEF animals exhibited greater WGA-HRP transport to the contralateral brainstem when compared to the ipsilateral side or to control animals. While previous experiments have shown that sparing and regeneration both occur with DCEF after contusion injury, these experiments show that DCEF is at least likely to promote axonal regeneration after axotomy in the spinal cord.


Unlike mammals, lower vertebrates are able to regenerate injured pathways of the CNS throughout life. In the optic nerve of goldfish, a good deal of research has been directed towards the changes in neuronal gene expression that accompany regeneration. However, the trophic factors responsible for initiating this process are unknown. We have shown that molecules secreted by the glial sheath cells and collected into culture medium (regenerative conditioned medium, RCM) induce neurite outgrowth in both embryonic mammalian cortical neurons (Caday et al., Mol. Brain Res. 45-50) and dissociated goldfish retinal cells (Bouls et al., these Abstracts, 1992). Conditioned media derived from other organs of the goldfish showed no similar activity.

Using the recombinant dye 4-d10-ASP, we have shown that RCM acts specifically on its natural target cells, the retinal ganglion cells, by promoting neurite outgrowth but not by influencing survival. RCM contains two trophic factors that differ in physical properties and patterns of expression. The smaller of the two, which accounts for most of the biological activity, is <1 kD in size, heat-stable, pronase and trypsin-insensitive, and has a hydrophobic domain. It is actively secreted and is expressed even in the intact optic nerve. The larger is a heat-labile, trypsin-sensitive, basic protein, Mr=70-100 kD, and is upregulated after axotomy.

Since these molecules are secreted from the optic nerve and target the retinal ganglion cells, they are likely to be important in inducing optic nerve regeneration in vivo. We are currently working to isolate the two factors and to evaluate the involvement of defined trophic factors in this system. Support: NIH EY 06690, HMMI, Boston Neurosurgical Foundation.


Apolipoprotein E (apo-E) is a lipid transport protein present in glial cells, whose concentration is increased following injury in most parts of the nervous system. The increase is due to synthesis by glial cells and to secretion by invading macrophages. An exception to this is in the optic nerve, where there is no increase in apo-E synthesis by glial cells following injury and there is a retarded rate of macrophage invasion. In addition, very little recovery or repair of the injured optic nerve occurs spontaneously. This study investigated the possibility that apo-E, administered systemically, might facilitate the repair or healing of the rat optic nerve following injury.

Crush injuries of the right optic nerve were made in anesthetized adult male rats. Apo-E purified from rat brain was injected into the nerve sheaths at the site of injury at varying doses (0, 1, 10, and 25ug). A retrograde tracing dye (biotinylated dextran conjugated to lissamine) was injected into the vitreous humor at 2, 6, or 13 days after injury. At 3, 7, or 14 days after injury, the nerves were harvested and prepared for immunohistochemistry to visualize: a) dye transport, b) macrophages, c) myelin. Apo-E administration affected several events associated with healing in a dose-related fashion. First, the transport of the dye was enhanced, both in distance past the injury and in the number of axons containing the dye. Second, the recruitment of macrophages to the crush site was significantly increased. Finally, the rate of myelin degeneration appeared to be increased. These data suggest that the presence of apo-E promotes several events known to be beneficial to nerve repair, and may provide an environment conducive to nerve repair or regeneration.

CHANGES OBSERVED FOLLOWING TREATMENT OF CHRONIC INJURIES OF THE BRAIN AND SPINAL CORD WITH AMP1 AND MATRIX. J.D. Peduzzi1, F.R. Fischer, and E.E. Geisert, Jr. 1Department of Physiological Optics, University of Alabama at Birmingham, Birmingham, AL 35294; 2Department of Anatomy and Neurobiology, University of Tennessee, Memphis, Memphis, TN 38163.

Regeneration is generally not observed after adult mammalian CNS injuries. Our efforts have been directed at manipulating the injury site by disturbing the scar and providing an environment favorable to axonal growth. Adult Sprague-Dawley rats received a brain lesion with a scalp blade or contusive spinal cord injury (10 g wt. dropped 5 cm) at the T9 vertebral level. After at least 2 months, animals received an injection into the injury site with one of the following: buffer, AMP1 antibody with EDTA, or AMP1 with EDTA and matrix. AMP1 antigen is a 110 kDa molecule that is upregulated at the scar. In the brain injured animals, AMP1 with EDTA appeared to disrupt the scar and growth cone-like structures were seen extending into the matrix. In spinal cord injured animals that received the AMP1 with EDTA and matrix, a slight but significant improvement was found in the combined behavioral score which is based on motor and sensory tests (modified from Gale, Keraédis and Wrathall, 1985, Exp. Neurol. 88:123). Supported by the Spinal Cord Society.


During the development of the central nervous system (CNS), the formation of axonal pathways and appropriate neuronal connections by axons is dependent on axonal growth and inhibitory interactions between cells and the extracellular environment. Proteoglycans are one of the many members of the extracellular matrix found to function in both the developing and mature CNS. One such proteoglycan, ABACKAN, was found in previous studies in functional boundaries of the developing rat CNS. In addition, ABACKAN was shown to inhibit both neuroblast cell attachment and neurite outgrowth on substrates of laminin. In the present study, ABACKAN was isolated from adult rat brain and preliminary biochemical analysis was performed to help determine the structure and possible function of the proteoglycan in the normal CNS. The proteoglycan was found to have an Mr of approximately 140 kDa and to be composed of a 170 kDa core protein to which both chondroitin and keratan sulfate chains are attached. A third carbohydrate moiety which was found to have HNK-1 antigenicity was also found attached to ABACKAN. To ascertain its function after injury in the CNS, the distribution of ABACKAN relative to specific cell markers and other proteoglycans was determined in normal and crushed optic nerve. ABACKAN, along with other proteoglycans, was found to be significantly upregulated after crush injury suggesting that ABACKAN plays a role in the inhibition of nerve regeneration. Supported by the Whitaker Foundation (EEG), NIH Grant # AR32666 (BC), the Canadian Spinal Research Organization (AS), and the NIH/NRSA Trauma Research Fellowship (DB).
545.11

EFFECTS OF IN SITU MICROINJECTION OF ANTI-NEUROFLAMENT MABS INTO REGENERATING GIANT CENTRAL NEURONS IN THE LAMPREY G.F. Hall, D.S. Pisac, E. Lampert* and M.E. Asen. Dept of Neurology, Children’s Hospital, Boston, MA 02115 and Dept of Neurology, Univ. of Penn., Philadelphia PA 19104.

Identified giant neurons (anterior bulbal cells or ABCs) in the hindbrain of the lamprey, a jawless sea lamprey. Permazol-injected brain have been shown to be an advantageous preparation for studying the effects of axotomy in situ using microinjection techniques. Here we examined the effects of microinjection of monoclonal antibodies to neurofilaments (LFM40) raised against the lamprey neurofilament protein (NF180) into regenerating ABCs. ABCs were injected with either of these mAbs at 4 mg/ml or 12 days following axotomy at the level of the 5th gill and were reaxonimized in the hindbrain at this time. Lucifer Yellow-dextran (LY-D, 10 mg/ml) was also injected to reveal the injected cells in wholemount. Injection controls consisted of rabbit IgG (5 mg/ml) and LY-D.

We found that anti NF180 mAbs injected a pronounced axon stump narrowing in treated neurons by 12 days post injection when compared to controls (p < 0.005, chi square test).

There was otherwise little difference between anti NF180 mAbs injected cells and controls in the shape of cell body and dendrites. In light of the role played by mammalian neurofilaments in maintaining axonal caliber, we propose that microinjection of anti NF180 mAbs blocks the transport of NF180 into regenerating axons. Supported by NIH grant NS29281 to G.F.H.

545.13


Oligodendrocyte inhibitory proteins retard axonal growth in mature mammalian CNS. We show that axons from 5 to 10 retina of hamster can show vigorous growth in the mature tectal environment, despite the presence of this target of differentoligodendrocytes. To test whether retinal neurons in retinal explants or animals have a capacity to regenerate the oligodendrocyte proteins, supernatant (30%) from hybridoma cells producing the neutralizing antibody In-1, or from control cells that produce an antibody against HRF, was added to co-cultures of retinal neurons in animals aged E14-15 and adult tadpoles. Cultures were maintained at 27°c for 5 days, fixed, and crystals of Dhl were placed in retinal explants to label the regenerating axons. In the presence of control antibody, the retinal axons were able to grow vigorously into tectum of all ages. In the presence of In-1 antibody, axonal outgrowth was significantly increased. Thus, E14-15 retinal neurons clearly have a receptor for the protein, and their growth by inhibition cannot be inhibited. In addition, we examined the effects of In-1 antibody in isochronic co-cultures prepared from animals aged P4 or older, when most retinal axons have lost the ability for axon elongation into the central target. We found that some retinal axons grew into tectal slices in an arborization mode at rate of about 100 μm per day. Addition of In-1 antibody increased the average number of axons entering tectal slices, but it did not change the basic mode of growth. The results suggest that factors intrinsic to the retina are involved in determining the growth ability of retinal axons, while myelinated inhibitors can limit the growth of retinal axons once they reach the CNS environment. (Support: NIH grants EY00126, EY01621.)

545.15

ALTERATIONS IN GLIAL MORPHOGENESIS AND ANTIGENIC PHENOTYPES FOLLOWING IRRADIATION. T.J. Sims, D.L. Davies and S.A. Gilmore. Department of Anatomy, University of Arkansas for Medical Scientists, Little Rock, AR 72205.

Early postnatal exposure of the lumbar sacral spinal cord to X-rays results in dramatic in vivo alterations in developing glial populations and in subsequent conversion to a CNS glial environment that is permissive for the regeneration of axons (Sims and Gilmore, Brain Res., 1994). To understand mechanisms involved in this conversion to a permissive environment, the status of the glial populations persisting after x-irradiation (40 Gy) of the 3-day-old spinal cord was further assessed in vivo by transgenic fate mapping. Control cultures prepared from non-irradiated spinal cord and experimental cultures established from the cords of irradiated littermates were compared by immunocytochemical and histochemical staining at 18 days in culture. The most conspicuous difference was the profound reduction in numbers of both astrocytes and oligodendrocytes in experimental cultures. In addition to differences in macroglia, the microglial population was severely depleted in experimental cultures (see Gilmore et al., this meeting). Five morphologically distinct astrocyte populations, both control and experimental cultures. The latter, however, contained a greater proportion of astrocytes with flat bipolar shapes. Addition of dibutyryl cyclic AMP (1 mM for 30 hrs) to control cultures released the cellular compartment to include greater proportions of process-bearing cell types, but failed to elicit a similar change when added to experimental cultures. Unexpected changes in phenotype were observed, however, such as astrocytes with cytoplasmic staining for glialactocerebroside, a marker normally associated with oligodendrocytes. This cell culture study strongly suggests that x-irradiation of the spinal cord not only reduces the glial populations, but also dramatically alters its cell composition and generates cells of distinct antigenic phenotypes. Supported by NIH grants NS 04768 and AA 07145.

545.16


Peripheral nervous system injuries often result in significant functional loss, including painful sequelae, that are often refractory to conventional surgical repair. The objective of the present study was to determine if a single application of the platelet-derived growth factor-BB (PDGF-BB)/insulin-like growth factor-I (IGF-I) combination could enhance the repair of injured peripheral nerve.

Forty-two rats were subjected to unilateral gap (8 mm) injuries of the sciatic nerve, and repaired with silicone tubes containing it the growth factors in a 2% methyl cellulose vehicle. Concentrations tested ranged from 15-700 μg/ml PDGF-BB and 30-160 μg/ml IGF-I, with metal ratios from 0.5:1 to 5:1 of PDGF-BF (N4-fold group). After 4 weeks, animals were evaluated by a ‘pinch test’ to demonstrate the extent of axonal growth, sacrificed, and biopsies were examined for the number of myelinated axons in the center of the tube and distal to the repair site. By these criteria, the PDGF-BB/IGF-I combination produced significantly greater nerve regeneration than treatment with vehicle alone across a broad range of ratios and concentrations. At the optimum metal ratio (1:4, PDGF:IGF), growth was doubled and 1470 myelinated axons (meansSD) compared to 1096±415 myelinated axons in animals which received vehicle alone. These data suggest that the combination of PDGF-BB/IGF-I might prove efficacious in the treatment of peripheral nerve injuries.
EFFECTS OF PRIOR NERVE INJURY ON MORPHOLOGICAL DIFFERENTIATION OF ADULT RAT DRG NEURONS IN CULTURE
Karen L. Lankford, Jeffrey D. Kocsis. Department of Neurology Yale University, New Haven, CT 06510.
Numerous studies have shown that conditioning lesions can enhance the rate of regeneration of subsequently injured peripheral neurons, but it is not clear whether this enhanced rate of nerve regrowth reflects an increased growth capacity of the neurons or changes in the growth environment. To assess whether conditioning lesions alter the properties of the affected neurons, we dissociated neurons from L4 and L5 DRG of adult rats subjected to spinal nerve crush 4-5 days before acute or chronic nerve ligation 3 weeks before sacrifice. Neurons were then cultured for 1-3 days, fixed, and stained with neurofilament antisera to visualize neurites. Rate of neurite initiation, neurite outgrowth, and branching frequency in previously ligated or crushed neurons were compared to neurons from control animals or the unoperated side of the same animal. Neurons extended processes at rates of several millimeters per day with interbranch distance being highly correlated with total outgrowth. Neurons which had previously received a crush showed roughly twice as many neurites with processes after 1 day in culture, greater average total neurite length per neurilemna bearing neuron, and increased interbranch distance compared with control neurons. Ligated neurons also initiated more neurites after 1 day than controls. These results demonstrate that enhanced regeneration of sensory neurons following a conditioning lesion is maintained in isolated cell bodies in culture.

CONTROL OF GAP-43 EXPRESSION CAN BE ACCESSED THROUGH A G-PROTEIN AND ADENYLYL CYCLASE
David J. Schreyer. Department of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.
Dorsal root ganglion (DRG) neurons display increased expression of a peripheral (but not central) axotomy in vitro. To study the signaling mechanism which links peripheral axotomy to changes in GAP-43 expression, we established a cell-ELISA technique to measure the GAP-43 content of microcultures of adult rat DRG neurons. As they do following peripheral axotomy in vitro, DRG cells which have been excised, dissociated, and maintained in vitro for one week display increased GAP-43 expression. This GAP-43 increase is partially suppressed by continuous exposure in vitro to cholinergic or dopaminergic agonists of the stimulatory G-protein G_3. The GAP-43 increase is also suppressed by the cyclic AMP analog dibutyryl-cAMP and 8-bromo-cAMP, the adenylyl cyclase activator forskolin, and the phosphodiesterase inhibitor Ro 20-1724. We propose that GAP-43 expression is normally suppressed by an external stimuli acting through G_3 and adenylyl cyclase, and that removal of this repressive action leads to the elevation of expression of GAP-43 which follows peripheral axotomy.

DYNAMIC REGULATION OF STRIATAL DOPAMINERGIC GRAPPS DURING LOCOMOTOR ACTIVITY
S. Hallett, 1, 2, H. M. Geller, 1, A. Testa, 1 and H. Nishino. 1, 2, 3.
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After ileal and colon grafts into hemiparkinsonian model rats, extracellular levels of DA increased nearly to normal and methamphetamine-induced rotations were reduced completely. However, more complex motor behaviors such as treadmill running at high speeds recovered to a lesser extent. Thus, besides recovery in tonic release of DA, additional mechanisms such as higher levels of D2ergic reinnervation under dopaminergic stimulation in the host brain might be necessary for better motor control. The present experiment was designed to estimate the neurochemical activity of D2ergic grafts during locomotion and to examine the functional importance of dynamic regulation of the grafted neurons in the host brain. Grafted rats were trained to run on a straight treadmill at various speeds (300, 660, 1200, 1800 cm/min), and extracellular DA and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid, were measured by in vivo microdialysis during and after running. Grafted rats were divided into two groups depending on the running ability (treadmill running test) and data were compared with those of normal and lesioned controls. Although tonic levels of extracellular DA in grafted rats recovered to 70-80% of control, those of DOPAC and HVA remained 15-20% of controls. Small numbers of grafted rats showed full recovery on treadmill running; percentage increases of DOPAC and HVA showed similar time courses and magnitudes to those of normal rats. The majority of grafted rats showed partial recovery of locomotion ability and percentage increases in DOPAC and HVA remained at lower levels than in normal rats. However, there were no differences in tonic levels of DA, DOPAC and HVA in the grafted groups. Data suggest that grafted D2ergic cells were dynamically regulated by the host brain and that the integrated release of DA might be involved in recovery of complex motor behaviors.

ORG 2766 ENHANCES AXONAL REGENERATION ACROSS TRANSECTED RAT SCRTIC NERVE
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Melatonin has been found to exert a trophic influence on both the central and peripheral nervous systems. The purpose of this study was to determine whether ORG 2766 (a tri-substituted ACTH(4-9) analog) would enhance recovery after complete transection of the rat sciatic nerve as determined by neuronal tracing techniques and twitch tension measurements.
Rates were anesthetized, the left sciatic nerve was cut in the mid-thigh, and the proximal and distal ends were inserted into a silicone tube and held in place with cyanoacrylate adhesive. The right sciatic nerve was left intact. For animals receiving ORG 2766 (N-V, O-gonon, The Netherlands) a 10 μg subcutaneous injection was given after surgery and 1 μg subcutaneous injections at 48 h intervals for a two-week period. After the eight-week survival period, twitch tension measurements were performed, followed by WGA-HRP injections into each nerve distal to the cut. After 48 h, the animals were perfused and the spinal cords were sectioned. The total number of labeled cells were counted from sections L2-S5 on both sides of the spinal cord and results were expressed as a ratio of labeled cells on the experimental side divided by the control side. The average of the ratios of labeled cells on the experimental (transected) side divided by the control (unoperated) side was 19.2% for the six animals which received injections of ORG 2766; three out of the five animals also showed the presence of twitch tension peaks recorded from the experimental side. The average of the ratio of labeled cells was 1.9% for the seven animals which did not receive any injections of ORG 2766; only one out of the six animals showed the presence of a twitch tension peak recorded from the experimental side. This preliminary study demonstrated that ORG 2766 did enhance recovery after complete transection of the sciatic nerve as evaluated by neuronal tracing techniques and also by twitch tension measurements. This research was supported by funds from the VA Rehabilitation R&D Center.

A MONOCLONAL ANTIHEURONAL ANTIBODY PROMOTES PRIMARY NEURON OUTGROWTH AND NON-NEURAL CELL SPREADING ON CNS MYELIN. AM LOZANO*, C. L. M. LARES and A. ROACH. Samuel Lunenfeld Research Institute, University of Toronto, Toronto, M5G 1X5.
A component of adult mammalian central nervous system (CNS) myelin inhibits axonal growth, a property that may be responsible in part for the long-term interruption of the CNS. The mechanism through which the inhibitory activity acts on the neuron to block growth is not known. To identify the neuronal molecules involved, we have generated and selected monoclonal antibodies based on their ability to promote neurite outgrowth on a CNS myelin substrate. We identified one such antibody, 10b, by its ability to promote the outgrowth of neurites from neural cell lines plated on CNS myelin. To test the effect of 10b on primary neurons and non-neuronal cells, we have grown newborn rat superior cervical sympathectic ganglion neurons (SCG) and 3T3 fibroblasts on CNS myelin. SCG neurons did not extend neurites and 3T3 fibroblasts did not spread on 10 μg/cm2 of CNS myelin. In contrast, on CNS myelin in the presence of 10b, there was a vigorous outgrowth of neurites from SCG neurons and extensive spreading of fibroblast cell processes. These effects were not seen with control antibodies. The results indicate that 10b influences the process through which myelin substrates exert their inhibitory effects on neuronal and non-neuronal cells.
546.3
THE EFFECT OF FETAL TISSUE GRAFT PLACEMENT ON BEHAVIORAL RECOVERY IN A RAT MODEL OF PARKINSON'S DISEASE
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Rats with unilateral striatal dopamine depletion are often used as an animal model of Parkinson's Disease. Fetal ventral mesencephalon grafts into the striatum of these lesioned rats have been used successfully to reduce asymmetries in both drug-induced and spontaneous behavior. We have found previously that grafts into the substantia nigra can also reduce the amphetamine-induced rotational bias. Thus, in the present experiment, rats were lesioned, grafted into either the striatum, substantia nigra, or into both. They were tested postoperatively for a reduction in bias of both amphetamine-induced rotation, and spontaneous home cage behavior. Fetal tissue was labeled with bromodeoxyuridine (BrdU). At the conclusion of the experiment, Biocytin was injected into the substantia nigra to trace the growth of the grafts. Coronal brain sections were stained for tyrosine hydroylase (Dsh), and Biocytin immunoreactivity. Trends in the data indicate that grafts into the striatum are the most effective in reducing drug-induced rotational behavior, followed by grafts into both sites simultaneously, and then grafts into the substantia nigra. Full data will be presented at the meeting.

[Supported by NS22157]

546.4

Grafts of embryonic dopaminergic tissue have shown promise as a therapy for Parkinson's disease in humans. One basic parameter debated in the amount of tissue necessary to achieve optimal behavioral recovery is to address the question: what the present experiment contrasted the effects of grafting ED15 ventral mesencephalic tissue (MESEN) from one (4d4) or four (16d) total donors into the striatum of adult male Sprague-Dawley rats with unilateral destruction of the mesencephalon dopamine system by 6-hydroxydopamine. Controls received equal volumes of ED15 spinal cord. Before grafting, the tissue was extended into 200 µm sections. Injections of either 1µl or 4µl/site were made for four sites into the striatum of each recipient. Circles after metamphetamine (MET; 5.0 mg/kg) was measured prior to grafting as well as one, two and three months to assess transplanted efficacy. Following the third time point the rats were deprived and trained to run on a circular treadmill for water reward, ipsi- and contralateral to the side of the grafting. Only the animals receiving 16d of MESEN showed a reduction in contraversive METH-induced turning, whereas those with 4d4 MESEN or 4 or 16 spinal glial did not show a reduction. A deficit in contraversive learned-turning was seen; however, none of the four treatments was effective in alleviating this deficit. Paradoxically, rats given 4d4 of MESEN showed the worst performance. Tyrosine-hydroxylase (TH) immunoreactivity revealed TH positive cells in the groups that received MESEN. We conclude that grafting with MESEN from four embryos produces a better behavioral outcome than from a single embryo.

546.5
THE STUDY OF APOMORPHINE-INDUCED ROTATIONAL BEHAVIOR IN PARTIALLY LESIONED RAT PARKINSONIAN MODELS WITH 6-HYDROXYDOPAMINE: Jin Woo Chang, Sang Sup Choong, Young Jin Kim*, & Kung Se Park, Seoul National University, Seoul 120-752, KOREA.

An apomorphine-induced rotational test has been used in the evaluation of rat parkinsonian model lesioned with 6-hydroxydopamine. We evaluated 50 partially lesioned rat parkinsonian models using the cylindrical rotometer devices with flat bottom (diameter, 33 cm). Depending on the pattern of the rotation induced by apomorphine (0s/kg, i.p., the rats were grouped into four groups: 1) contraversive rotational, 16 cases: Group 1: 2) converversive rotational, 20 cases: Group 3: 3) ipivertive rotational, 9 cases: Group 4: 4) non-preferred or no rotation, 11 cases). Twelve of 16 rats in group 1 and 14 of 20 rats in group 2 met our criteria of rat parkinsonian model (at least 90 turns/30 min, 10 min after apomorphine injection). Although another 4 rats in group 1 did not meet our criteria, all of them showed severe losses (more than 95%) of the TH-immunoreactive neurons (SNpc) in the lesioned side. Another interesting finding was that rats in group 2 and 3 showed moderate losses (50-90%) of TH-immunoreactive neurons (SNpc) in the lesioned side even though their rotational responses were different. These results suggest that pattern of rotational (ipsiversive rotation) is a more reliable index of neuronal losses of SNpc than the total numbers of the rotational response to apomorphine.

The exact cause of the abnormal ipiversive rotation noted in group 3 is unclear. Further research should be pursued to explain this finding.

546.6

We examined a model of Parkinson's disease to test the effect of embryonic grafts of ventral mesencephalon on motor disability induced by MPTP. DA increases on average 500% near grafts (see Elsworth et al.). Although staining revealed 20,000+ DA neurons in grafts derived from a single embryo, we have seen variability in the percentage of DA neurons that survive the grafting procedure. This has led to the question of the donor tissue; neurons that have extended axons would be subject to degeneration while cells taken from younger ages prior to neurogenesis may represent a more appropriate developmental class. Grafts were selected from pregnant monkeys by autoradiography; 14 donors were chosen from embryonic days 34-74. Solid tissue grafts were placed into the caudate nucleus. Brains were collected at 3-6 months after surgery and were stained for tyrosine hydroylase (TH). DA neurons ranged from 1,300 to 28,000 per donor; 5 of 14 animals contained between 8,200 and 28,400 TH neurons. The numbers of TH neurons in grafts seen at each age were: E31 (3,500), E38(13,780), E92(28,908), E121(21,296), E142 (4,120), 1,300, 1,900, 11,700, 12,800, 14(404), 1,410, 15,964, 28,400, E170(1,200), 13,344. The average of donors (n=11) taken during postnatal neurogenesis (E36-E45) is 24,870 ± 2400. of the substantia nigra is 12,900±256. The adult African green monkey has about 80,000 DA neurons in both substantia nigra. Thus, an average survival of 12,500 neurons is about 16% of the adult population; the best survival obtained (i.e., E28) is about 36% which is substantially better than results obtained from studies using xenograft human cells. Supported by NIH grants NS 24032 and RSA MH 06453 (DER).

546.7

Occasional studies in pallimcanine animals have suggested that the mechanism of neural transplant-induced beneficial effects may not rely on dopamine (DA) generated from the neural graft. The present studies address this issue in MPTP-treated monkeys. Grafts of fetal ventral mesencephalon (VM) or cerebellum were placed stereotactically in the striatum of MPTP-treated parkinsonian monkeys. Behavior of each animal was assessed before and following studies. As a precautionary measure, tissues were removed from brain slices for biochemical analysis. The removal of the slice was processed for autoradiography or tyrosine hydroylase (TH) immunohistochemistry. Striking elevations of TH in dopamine containing DA, were observed in the growth of transplanted 2.5 cm CRIL fetal VM cells. High density of binding was seen at the site of the graft. Graft presence was confirmed by TH immunohistochemistry. At some further maturation, an increased density of binding to DA uptake sites. Correlative in vivo brain imaging by SPECT has been performed in some grafted MPTP-treated monkeys using [123I]r-CIT. Supported by NS 24032, the Asian Research Foundation, RSA MH 0043 to DER.

546.8

Fetal mesencephalic grafts into the striata of partially dopamine (DA)-depleted African green monkeys become well developed and show numerous TH-positive neurons when examined with immunohistochemistry. These neurons project fibers into the adjacent host stria. Since control of DA synthesis/release may be influenced by the host brain, the establishment of reciprocal connections is of importance. A double label IHC staining technique was used for simultaneous evaluation of both graft and host fiber projections. TH-containing neurons were stained as before. Endogenous host TH-positive fibers were identified by the immunohistochemical reaction of the TH-positive graft that contained neuronal GABA and substance-P. Different chromogens were used to label the different cell types. This staining protocol was performed on brains obtained from three animals. Positive staining for TH substance-P-Fibers was identified within the TH-positive grafted fetal mesencephalic tissue. Many of these fibers were found in proximity to TH-positive graft cells. Some of the fibers adjacent to TH-positive cells exhibited enlargements suggestive of potential contact points. These preliminary results provide a morphologic basis for the presence of reciprocal connections between the host striatum and the transplanted fetal mesencephalon. Electrophysiological and electron microscopic studies of an analogous experimental paradigm will be needed to verify this suspected connectivity. Supported by a Chonliul Brain Research Fellowship, Axiom Research Foundation, and NIH grants NS 24032 and RSA MH 06643 to DER.
546.9
FUNCTIONAL RECOVERY IN HEMIPARKINSONIAN MONKEYS FOLLOWING GRAFTS OF ENCAPSULATED PC-12 CELLS 1-2,3,4,5,6,7,8,9,10,11
Dopamine, which control all ChAT-(0-36%) grafts.

STRIATAL SECRETS OF NGF: BHK-hNGF FOR PC-12
These cells recovered their use of the affected limb and performed at near-normal levels. The third PC-12 graft monkey had the one control monkey remained intact. These monkeys the ChAT-ir striatal

546.11

Six aged Rhesus monkeys (24-29 years old) received unilateral transplants of the three groups that received NGF-secrating cells displayed only a modest loss of ChAT(0-36%) and p75 NGFR-immunoreactive (ir) (53%) medial septal neurons ipsilateral to the lesion/graft. In contrast, monkeys receiving grafts of encapsulated NGF-secreting cells displayed only a modest loss of ChAT(0-36%) and p75 NGFR-(7-22.4%) striatal neurons. Additionally, all monkeys receiving the NGF-secreting implants, but none receiving control implants, displayed robust sprouting of cholinergic fibers within the septum ipsilateral to the transplant. Just prior to sacrifice, the capsules were retrieved and determined to contain viable BHK cells releasing biologically relevant levels of NGF. These data demonstrate that hNGF can provide trophic and trophic influences to degenerating cholinergic basal forebrain neurons in aged nonhuman primates supporting the contention that hNGF may prevent degeneration of basal forebrain neurons in Alzheimer's disease.

546.12

A common feature of aged Rhesus monkeys (24-29 years old) was reduced levels of useable hNGF. The third PC-12 graft monkey in which behaviorally recovered normal striatal function was noted in November. 1992. The recovery of the striatum, functional recovery in the two PC-12-grafted monkeys was sustained for up to 2 months. These data suggest that grafts of PC-12 cells can induce functional recovery in nonhuman primates although the mechanism of action remains unclear.

546.13
HYPERTHYROID OF CHOLINERGIC AND NPY CONTAINING STRIAL NEURONS FOLLOWING IMPLANTS OF POLYMER ENCAPSULATED CELLS GENETICALLY MODIFIED TO SECRATE HUMAN NGF. E.Y. Chen 1, D.P. Emerich 2, S.R. Wimg 1, E.L. Freedel 1, J.P. Hammond 2, E.E. Baste 2, and J.H. Kordower 1.

The present study examined the effects of polymer encapsulated grafts containing baby hamster kidney fibroblasts (BHK) genetically modified to secrete human NGF (hNGF) on the size of cholinergic and noncholinergic striatal neurons following implantation into the intact rodent striatum. Three groups received BHK-hNGF grafts into the right striatum and were sacrificed 1, 2, or 4 weeks post-implantation. The remaining three control groups were treated identically except that BHK cells were not modified to secrete hNGF. In control BHK graft rats, the size of ChAT-ir striatal neurons was unchanged (c6%) at all time points. The BHK-hNGF grafts, there was a hypertrophy of ChAT-ir striatal neurons. These data suggest that polymer encapsulated cells which were genetically modified to secrete hNGF can influence both cholinergic and noncholinergic neurons within the striatum.

546.14
CLINICAL RECOVERY AND GRAFT SURVIVAL IN PARKINSONIAN NON-HUMAN PRIMATES AFTER STRIATAL GRAFTING OF FIBROBLASTS GENETICALLY MODIFIED WITH TYROSINE HYDROXylASE (GDNF). K.S. Banks 1, A. Eason 1, D.A. Vuye 1, A. L. Maud 1, K. Sofat 1, G. Choudry 1, M. W. McNaghten 1, M. D. Scott-Wright 1, and D. A. W. Cork 1.

Somatic gene therapy uses grafts of genetically modified autologous cells to produce therapeutic substances within the body, and eliminates the need for immunosuppression or barrier devices. We are utilizing a process that consists of harvesting autologous cells through a biopsy, carrying out ex vivo genetic modification of the therapeutic, genetically active proteins, and subsequently grafting these to an appropriate site. 7 MPTP-treated, overexpressed hemiparkinsonian monkeys were used for this study. Using MRI-guided stereotoxic surgery the 3 monkeys were grafted with autologous fibroblast transfused with retrovector (hFGS) carrying a human tyrosine hydroyxylase (TH) cDNA. 2 monkeys were implanted with non-transfected fibroblasts and 2 animals were left non-implanted. Prior to the implantation all animals were behaviorally characterized for 4-6 months using using the following scales: apomorphine-induced turning, arm use scale and activity monitors. TH implanted monkeys showed immediate clinical improvement and increase of activity after the implantation which lasted for at least 6 months. The grafted fibroblasts showed the ex vivo transplantation and the mediated functional arm use. Animals were sacrificed at 4 months. Control implanted animals recovered only from amphetamine-induced rotation, while control non-implanted monkeys remained unchanged. MRI scanning detected grafts in the striatum and closure of the blood brain barrier at 2 weeks post-implantation.

Histological examination of hemiparkinsonian non-primate 1 (13%) 2, 16% (13%) and (13%) weeks post-implantation. Removal of the capsules just prior to sacrifice revealed healthy BHK cells which secreted sufficient levels of hNGF to differentiate PCs. These data suggest that polymer encapsulated cells which were genetically modified to secrete hNGF can influence both cholinergic and noncholinergic neurons within the striatum.
546.15
Administration of 0.4 mg/kg of MPTP into one internal carotid artery produces hemiparkinsonism in monkeys. In such a model, the side ipsilateral to MPTP administration is typically depleted of DA, while the side contralateral serves as a "normal" control. As we previously reported (Bankiewicz KS, et al, Life Sci, 1986), unilateral exposure to 0.8 mg/kg results in overexpressed HPD monkeys which display bilateral signs of the PD. In this study, we further characterized HPD+ monkeys using behavioral and in vivo biochemical methods. 17 Rhesus monkeys (age 5-16, weight 3.52-14.86 kg; 6 females, 11 males) were studied during 5-18 months after MPTP injection. The monkeys received an intracranial infusion of 2.4-μg MPTP solution 0.34 mg/kg to induce HPD+, which still enabled them to sustain their spontaneous turning. Asynchronous-induced turning, arm use, general activity measurements, clinical recordings using rating scale, in vivo microdialysis and CSp levels of HVA were examined. After the MPTP treatment, all of the monkeys showed progressively bilateral signs (freezing, bradykinesia, decreased defense reaction, posture and gait problems), and hemiparkinsonian signs with predominant use of the contralateral arm, and decreased ipsilateral spontaneous circling and contralateral-induced contralateral turning. The CSp HVA levels were increased in the first hours after the MPTP administration with subsequent decrease of 40-65% 3 days later. Extracellular levels of dopamine and HVA as measured by microdialysis in caudate and putamen nucleus on the ipsilateral side were not detectable. Local amphetamine administration induced DA and HVA peaks only on the contralateral side, suggesting total lack of any remaining innervation on the ipsilateral side. This side, modelling the end stage of human PD, could be used for grafting DA-producing cells for studying DA replacement therapy, while the partially lesioned contralateral side, modelling an advanced stage, could be used for studying trophic factor-releasing cells and inducing a trophic reaction.

546.16
CCK FACILITATES METHAMPHETAMINE-INDUCED Dopamine OVERFLOW IN RAT STRIATUM AND FETAL NIGRAL GRAFTS. Y. Wang* and S.L. Peng. Dept. of Pharmacology, National Defense Medical Center, Taiwan.
The purpose of this study was to investigate the electrochemical interactions of cholecystokinin (CCK) and methamphetamine (MA)-induced dopamine (DA) overflows in rat striatum. High-speed chronocompartmental recording techniques using Nation-coated carbon fiber electrodes were used to evaluate extracellular DA concentration in the striatum. CCK-AS, CCK-8S, MA and DA were locally applied directly to the striatum of urethane-anaesthetized Sprague-Dawley rats. We found that CCK potentiated MA-induced DA release in the anterior striatum. This reaction is likely mediated through CCK-A receptors because only CCK-AS, but not CCK-8S, enhanced MA action. Replacement of Ca2+ with Mg2+ antagonized this reaction, suggesting that the modulation of MA by CCK is Ca2+ dependent. Both MA-induced DA release and CCK modulatory effects were disappeared in the striatum after unilaterally lesioning the medial forebrain bundle with 6-OHDA lesions. The unilaterally lesioned rats were later transplanted with fetal ventral mesencephalon. We previously found that the zone of normalized dopamine clearance was considerably larger than that of normalized release in the anterior striatum after fetal nigral transplantation, which may be contributed to the partial reinnervation from the transplant. In the present study, we found that the modulation of DA release by CCK was regenerated in the zone of normalized release after fetal nigral transplantation. However, CCK did not increase MA-induced DA release in the partially uninnervated area. In conclusion, these findings suggest that the not only DA release process but also CCK modulatory mechanism were regenerated after fetal nigral transplantation.

546.17
Reynolds and Weiss (Science 255:1707; 1992) reported the isolation and partial characterization of epidermal growth factor (EGF) responsive striatal progenitor cells. Using their procedure, we have isolated mesencephalic progenitor cells. E14.5 rat mesencephalon was isolated and plated onto 100 x 20 mm petri plates (1200 cells per cm2) that had not been pre-treated with extracellular matrix products. The cells were grown in media containing 20 mg/ml EGF. Less than 0.1% of the cells survived and went on to form clusters of cells if left undisturbed for 14 days. These mitotically active cells have been successfully passaged for 8 months. When cultured on poly-L-lysine coated plates containing a complete media (10% FCS), the cells differentiated into glia and neuron-like cells. None of these cells stained positively for tyrosine hydroxylase. Similarly, TH positive cells were not found after exposure to various neurotransmitters such as DA, 5-HT, NE and EPI. In contrast, co-culturing progenitor cells with fresh mesencephalon had a profound effect increasing the number of TH staining neurons (FP = 0.01) relative to mesencephalon cultures. Mitochondrial dehydrogenase activity was unchanged in the co-cultures suggesting that mitotic activity was not responsible for the increase in TH neurons observed. The cultures were also negative for the NE marker DBH. These data suggest that a soluble or cell contact mediated activity present in mesencephalon was responsible for converting the progenitor cells into a DA neuron phenotype. Like striatal cells, mitotic progenitor cells capable of developing into DA neurons exist within the mesencephalon.

546.18
The basal ganglia exert their function mainly via disinhibitory GABAergic circuits. However, very little is known about the changes that occur in GABA neurotransmission in Parkinson's disease, and virtually nothing is known about such changes following dopamine supplementation by grafted fetal dopaminergic neurons. In the present study, GAD mRNA levels were compared in neurons of the entopeduncular nucleus (EPN) and the substantia nigra pars reticulata (SNR) in normal, unilaterally 6-hydroxydopamine-lesioned and grafted rats by means of in situ hybridization using 35S-labeled cRNA probes. Autoradiographs revealed a moderate elevation of GAD mRNA levels in the ipsilateral SNR, particularly in the lateral half of the nucleus, while the ipsilateral EPN showed a slight increase. Nine months after transplantation of fetal nigral neurons, the GAD mRNA levels remained significantly elevated in the ipsilateral SNR, but the ipsilateral EPN appeared similar to those of normal rats. These data indicate that nigral grafts placed in the striatum differentially influence GAD mRNA levels in the EPN and SNR, and suggest a necessity for additional dopaminergic grafts in the nigra for complete restoration of basal ganglia function. [Supported by the Parkinson's Foundation of Canada].

Fast axonal transport (FAXT) is responsible for the bidirectional movement of proteins within the nerve terminal and axon. Anterograde transport of FAXT of neurotransmitter-associated proteins in the magnocellular organellar membranes to nerve terminals occurs at a rate of ~410 mm/d. Pulsed electromagnetic fields (PEMF) have been shown to enhance axonal outcome with sciatic nerve when applied before or after injury, although the mechanism of this effect is not known. In this study, we examined the effects of acute PEMF treatments on fast axonal transport in sciatic nerve and sciatic-nerve crush (Sprague-Dawley) rats under 2 conditions: in unjured (non-operated) nerves and 1 d after a crush of the nerve behind the knee. Rats were placed between Helmholts coils for 15 min/for 2 d preceding injection or for 60 min on the day of injection. While maintaining core body temperature at 37°C, [³H]-proline was stereotactically microinjected into the spinal cord; rats were killed 2.5-5.0 hr later. After tissue solubilization and liquid scintillation spectroscopy, transport profiles indicate that acute treatments with PEMF had no effect on fast transport rates in injured or unjured sciatic nerves. These studies are in agreement with others indicating that fast axonal transport rates are not altered in response to crush injury. They also show that PEMF effects are not related to transport mechanisms. Slow axonal transport using a similar paradigm are underway. Supported by NIH NS 29621-02 (BFS).

The diffusible fast axonally transported DRG proteins do they represent common or unique secretory products? B. Tedeschi, S. Mulugeta and R.P. Chavara. Departments of Anatomy & Neurobiology and Microbiology & Immunology, East. Virginia Med. Sch., Norfolk, VA 23501.

Fast axonal transport (FT) is believed to represent the translocation of vesicles/shapes in the neuronal secretory pathway. In the frog DRG/sciatic nerve preparation, we have previously demonstrated the diffusible FT protein subset from the membranous organelle sub-fraction. In the present study we addressed two questions: (1) Which diffusible FT proteins are unique relative to the secretory products of non-neuronal nerve cells? and (2) Which diffusible FT proteins are unique relative to other neuronal FT proteins? Neuronal or non-neuronal nerve protein pulse-labeled with -S-methionine and time allowed for FT (in the case of non-neuronal proteins). Radiolabeled diffusible proteins were collected in a bath surrounding nerve and bath/nerve proteins were subsequently analyzed by two-dimensional gel electrophoresis (2D-PAGE) and fluorography. Results showed that: (1) only one (10%) of the diffusible DRG FT proteins exhibited a unique identity with a diffusible non-neuronal sciatic nerve protein and (2) all of the diffusible DRG FT proteins were FT by retinal ganglion cell neurons. These results suggest that diverse neuronal populations may package a similar set of soluble FT proteins and that these diffusible products are quite different from the secretory products of non-neuronal nerve cells.


rab3A and B belong to the ras-related superfamily of small GTP-binding proteins and are mainly present in nervous and endocrine tissues. These proteins appear to be involved in the control of a late step of exocytosis during which vesicles become docked to the plasma membrane. Physiological studies showed that these proteins may differentially regulate tonic and phasic exocytosis. This data prompted us to examine the effect of addition of a mRNA encoding rab3A and B. In situ hybridization revealed that both mRNAs are heterogeneously distributed in the brain but overlap in most areas, although rasB3B-mRNA is generally less abundant, except in the pituitary and olfactory bulb. This distribution was quantitatively ascertained by competitive PCR in the same tissues. In addition, double in situ hybridization showed that rasB3A and B mRNAs are expressed within individual neurons. During lactation, both rasB3A and B mRNAs are upregulated in the magnocellular hypothalamic system in which increased stimulations are associated with the pulsatile release of oxytocin from the neurohypophysis. During lactation, an increase of rasB3B mRNA was also observed in the anterior pituitary. Western blots confirmed these results and revealed the rasB3A and B proteins exhibiting apparent molecular weights due to a post-translational modification.

RIBOSOMES BEARING SHORT GAP-43 NASCENT PEPTIDES BIND TO NUCLEAR AND MICROSPERICAL SUBCELLULAR FRACTIONS. R. Moore-Batchelder, H. Pentama, and J. Denney*. Division of Life Sciences, Univ. of Texas at San Antonio, San Antonio, TX 78249.

We have transcribed a linearized pGEM3 vector containing a full-length GAP-43 cDNA insert and have translated the purified, uncapped mRNA in vitro using reticulocyte lysate and 35S-methionine. A nuclear fraction isolated from cultured PC12 cells and a microsomal fraction isolated from either PC12 cells or from canine pancreas were added to translation mixtures both co-translationally and post-translationally at 30°C. The canine microsomes were specifically added in the co-translationally experiment fractions were present during a 1 hour translation that was subsequently blocked either with cycloheximide or puromycin/high salt. In the post-translationally experiment fractions were incubated with mixtures for 1 hour after the translations had been blocked with cycloheximide or puromycin. Under these conditions, we observed an enrichment of GAP-43 nascent peptides of molecular weight 22 kDa and below in high-speed Airfuge pellet fractions relative to all larger nascent peptides and full-length GAP-43 and also relative to pellets obtained from translation mixtures that did not receive any subcellular fraction. These results suggest that short GAP-43 nascent peptides are competent for interaction with membranes while longer peptides are not.
547.7
Several subunits and their isoforms have been identified encoding the γ-aminobutyric acid receptor (GABAR). Assembly of specific subunits leads to different pharmacological properties and cell surface localization. Despite the potential to assemble both homo- or heteromeric receptors in nonneuronal cells, there is emerging evidence that assembly and expression of GABAR subunits in neurons may be restricted. We have examined the sorting, cell surface expression, and localization of the α1 and β3 GABAR subunits in PC12 and COS-7 cells for clues of how receptor assembly of specific GABAR subunits affects localization and membrane mobility in neurons. PC12 cells transfected with cDNA encoding the α1 subunit retain the α1 subunit in an intracellular compartment whereas PC12 cells transfected with GABAR encoding the β3 subunit sort it to the plasma membrane and form clusters on both cell bodies and processes. After transfection of both α1 and β3 in PC12 cells, both proteins are found on the cell surface co-localized in clusters. In contrast to oocytes and epithelial cells, neuron-like cells express at their plasma membranes a more restricted repertoire of GABAR complexes. Moreover, we show that the localization of GABAR in discrete domains can be conferred by the β3 subunit. Cos-7 cells, used to study the intracellular localization at higher resolution, sort GABAR subunits similar to PC12 cells. The location of α1 in Cos-7 cells is consistent with an ER pattern suggesting that α1 may contain an ER retention signal. The β3 subunit, able to redirect α1 from the intracellular compartment to the cell surface, was not able to rescue a truncated α1 containing only the first two transmembrane domains. These results suggest that neurons likely have a mechanism by which specific complexes are assembled, requiring all the transmembrane domains, and architecturally edited before expression on the cell surface. Supported by 2F2NS09198-03 and NS0872.

547.8
SORTING AND CELL SURFACE EXPRESSION OF NMDA RECEPTOR SUBUNITS
Ihsana Makal-Jeticovic, Leislhona Karayiati* and Himon J. Angellides, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.
Neurons are highly polarized cells with a complex morphology, where proteins are targeted to discrete regions of the cell surface. At synaptic sites N-methyl-D-asparate receptors (NMDAR) appear to be clustered at high density mediating the events of glutamate neurotransmission. Molecular cloning has identified several cDNAs encoding NMDAR subunits NR1, NR2A, NR2B, NR2C and NR2D. The NR1 subunit is essential in a heterologous configuration for electrophysiological and pharmacological characteristics of NMDAR. There is emerging evidence that the subunit composition of ligand-gated channels can determine sorting and targeting of the receptors. In order to study how neurons process NMDAR we have transfected COS-7 and neuron-like PC12 cells with cDNA encoding the NR1 subunit. We have used subunit specific antibodies, indirect immunofluorescence, and light and confocal microscopy to determine the cellular distribution of the NMDAR subunits in transfected cells. In COS-7 and PC12 cells NR1 is retained intracellularly when expressed alone. The majority of NR1 protein is sequestered in the Golgi compartment and a small fraction is found in lysosomal vesicles. When transfected cells were treated with Brefeldin A to dissociate the Golgi apparatus, NR1 was redistributed into the ER. The presence of NR1 beyond the ER in transfected COS-7 and neuron like PC12 cells strongly suggests that the protein is folded appropriately and has been processed into a transport competent configuration. The NR1 subunit was not detected on the surface of those transfected cells by immunofluorescence or immunoblotting of biotinylated surface proteins. These results raise the possibility, that the presence of more than one subunit is necessary for cell surface delivery of functional NMDAR and expression of the complex is regulated by availability of the other NMDAR subunits. Supported by grant NS28072 of HSPD.

547.9
There is a great deal of interest in studying the cell biology of neurons. However, because neuronal cell lines have been difficult to obtain and because large cultures of purified primary neurons are difficult to prepare, many standard cell biological studies have not been feasible. Furthermore, it is often difficult to express foreign genes in neurons. In an effort to overcome these disadvantages, we have characterized vaccinia virus (VX) directed expression of foreign proteins in the human cell line NTERA 2 (NT2). Using retinoic acid, NT2 cells differentiate to form large, highly purified (99.9%) cultures of mature neurons (NT2-N). We found that NT2-N cells are readily infected by recombinant VY, yet they maintain their polymorphic morphology and die at a rate matching non-infected controls. To determine if foreign proteins can be expressed and properly processed in NT2-N cells they were infected with recombinant VY expressing the HIV-1 glycoprotein, gp160, under the control of a VY early/late promoter. Gp160 was easily detected 1 day post-infection by western blot, pulsed-labeling, and immunofluorescence microscopy. Gp160 was transported to the Golgi apparatus, efficiently cleaved into gp120 and gp41 subunits, and expressed on the cell surface. Despite efficient expression the infection was non-productive (virus particles were not produced).

The ability of NT2-N neurons to efficiently express foreign proteins, with few of the cytopathic responses normally associated with VY infection, provides a novel opportunity to address a wide array of cell biological questions in a system of pure human neurons.

547.11
ENTEROPATHOGENIC ESCHERICHIA COLI DEPOLARIZES INFECTED HELA CELLS. H. Yan, D.A. Mathers*, M.A. Stein and B.B. Finlay. Departments of Physiology and Biotechnology, University of British Columbia, Vancouver, BC Canada V6T 1Z3.
Enteropathogenic Escherichia coli (EPEC) causes severe diarrhea in children. EPEC attach intimately to gut intestinal epithelial cells and mediate cytoskeletal rearrangements characterized by the formation of pedestal-like structures and localized degeneration of brush border microvilli. We have applied patch clamp methodology to characterize the electrical events occurring during the early interactions of EPEC with epithelial cells. HeLa epithelial cells were incubated with EPEC strain E2348/69 for 30 minutes at 37°C and washed extensively. Cells with attached EPEC colonies were used for whole-cell recordings, made at 21-24°C with patch electrodes containing (in mM): 135 KCl 5 NaCl 1 MgCl2, 10 HEPE and 5 NaF. Cells were washed, E70 buffered in a saline containing (in mM): 135 NaCl 4 KCl 1.8 CaCl2, 1 MgCl2, 10 HEPE and 5 glucose. The mean resting membrane potential (VREST) of HeLa cells infected with EPEC was -24 mV. Cells incubated with supernatant from EPEC cultures, normal culture media or an EPEC mutant (eaeA-2-1) that does not mediate cytoskeletal rearrangements, showed mean VREST values of about -48 mV. The results suggest that membrane depolarization is an important component of the pathogenicity of EPEC.
548.1 NAPDH-DIAPHORASE HISTOCHEMISTRY OF RAT CHOROID PLEXUS. A. Chodobski, J. Szemydor- Chodobski, P.R. Moftsils, A.Y.I. Liu, M. Evensen, and C.E. Johnson. Program in Neuroscirnomy, Department of Clinical Neurosciences and Central Research Laboratory, Brown University/R.I. Hospital, Providence, RI 02903.

NAPDH-diaphorase (NAPD-d) has been frequently co-localized with neuronal isoform of nitric oxide synthase (NOS), a synthetic enzyme for nitric oxide (NO), and is, therefore, considered as a histochecmical marker of the neuron. In the present study, we demonstrate the colocalization of NAPD-d-positive staining with NOS in rat choroid plexus (see accompanying abstract by J. Szemydor-Chodobski et al.). In transcardially perfused rat brains with 4% paraformaldehyde and brains were postfixed for 4 h at 4 °C. Parts of the choroidal tissue were dissected out and incubated at 37 °C for 3 h in PBS (pH 7.6) containing 1 mM of nitrile blue tetrazolium and 1 mg/kg of NAPDH. Triton X-100 was added (0.6%) to improve tissue penetration. NAPD-d-positive nerve fibers were found to accompany anterior choroidal artery and its branches, as well as smaller arteries within the choroidal stroma. Choroidal veins and venules were devoid of NAPD-d staining; however, larger veins draining blood from the inferior part of the choroid plexus, proximal at the basal vein, had NAPD-d-positive innervation. Endothelial cells were stained as well. In addition, a deposition of the reaction product (formazan) was found in the cytoplasm of choroidal epithelial cells, with nuclei being free of staining. In conclusion, NAPDH-d was shown to be colocalized with NOS in the choroid plexus. The presence of NAPD-d in different choroidal cell types suggests the multiplicity of metabolic barriers in mechanistic changes in choroidal tissue. Supported by NIH Grant NS 27601.


We have previously shown that nitric oxide (NO) mediates some hemodynamic properties of angiotensin II (Barron et al., in press). In the present study, we demonstrate by immunohistochemistry the presence of NO synthase (NOS), a synthetic enzyme for NO, in rat choroidal tissue. Anti-NOS antibody was raised in rabbits against the brain NOS. It recognizes neuronal NOS in several mammalian species, including the rat, and does not cross-react with inducible NOS. Rats were perfused transcardially with 4% paraformaldehyde and brains were postfixed at 4 °C. Parts of the choroidal tissue were dissected out and incubated for 48 h at 4 °C with primary antibodies (1:2000). The procedures which were performed were performed at room temperature. Biotinylated donkey anti-rabbit IgG was used to detect primary antibody, followed by incubation with streptavidin-biotin-peroxidase complex. To obtain color reaction, diaminobenzidine was used. NOS-positive nerve fibers were found to be associated with the anterior choroidal artery and its branches, as well as with smaller arteries located within the choroidal stroma. Choroidal veins and venules were devoid of NOS-containing innervation. Endothelial cells were not stained, indicating that the antibody does not cross-react with endothelial form of NOS. Choroidal epithelial cells expressed intense, distinctive staining of their cytosol. These results provide anatomical evidence supporting the physiological observations of all inhibitors of NO activity in different tissues and their NOS-containing components. We are currently investigating the relationship of NO activity in the choroidal circulation and its pathophysiologic implications. (Supported by NIH Grant NS 27601 and by research funds from the R.I. Hospital.)

548.3 GLOSOMATIC OPENING OF BLOOD-BRAIN BARRIER TO ENDOGENOUS ALBUMIN. A.W. Vorbrodt, D.A. Dobrovolnaya, and A.S. Lagunov. NICGNS for Basic Research in Developmental Disabilities, Staten Island, NY 10314. Early observations of the blood-brain barrier (BBB) to endogenous albumin were studied using a new quantitative immunocytochemical procedure, hypercrosslink Li+ arabinose solution (1.8%) was infused into carotid arteries of animals, (which were killed 5 and 30 min afterwards. Brain samples were immersion-fixed and embedded at low temperature in Lowcryl K-4M. Ultrathin sections were exposed to anti-rat albumin antiserum followed by protein A-gold. The density of immunosignals (gold particles per μm²) was registered and counted over four compartments: vascular lumen, endothelial cells (ECs), subependothelial space (including basal lamina (BL), and the adjacent astrocytic processes with hypermorphic NOS). The labelling density of the vessel lumen was considered to represent 100% of the signal, other compartments were corrected to that level. On quantitative analysis indicated that in control animals, only 0–4.5% of the circulating albumin appears in the subependothelial space, whereas as soon as 1 min after infusion, this value increases to 3% followed by a further increase to 5% at 15 min respectively. The main routes of albumin escape are through the damaged EC cytoplasm (including vesicles and penetrating indentations) rather than through modified interendothelial junctions. A slow increase of immunosignals in the adjacent neuropil suggests that the BL represents a serious obstacle for escaping albumin which seems to get trapped in this structure. (Supported by the National Institute on Aging, grant RO-AG 10279–03).

548.4 EFFECTS OF HYPERTONIC SALT INFUSION ON CEREBRAL PERFUSION IN NORMOTREMATIC AND HYPERTREMATIC RATS. S. Adler, D. Williams and J.C. Verbalis. University of Pittsburgh School of Medicine and Carnegie Mellon University, Pittsburgh, PA 15213. Rapid increases in plasma Na (pNa) and osmolality can disrupt the blood brain barrier (BBB). Recent studies from our laboratories have shown that this occurs at lower thresholds in hypertensive (HN) than in normotrematic (NN) rats. The present experiments studied the effect of similar increases in pNa on cerebral perfusion in NN and HN rats. NN (n=6), acute HN (n=8), and chronic HN (n=10) rats received iusions of either 700 μL/kg 500 mOsm NaCl. Both cortical and white matter perfusion were measured by arterial spin tagging in a 4.7 Tesla NMR magnet before and at 30, 60, and 90 min during the infusion. pNa increased 22%, 20%, and 19%, respectively, and arterial pressures remained constant throughout. Arterial pCO2 rose slightly in all groups (from 61 to 63 in NN, 57 to 64 in acute HN, and 47 to 48 mm Hg in chronic HN). Cerebral perfusion increased significantly in both regions in the HN rats at all times. For example, by 60 min perfusion had increased in cortex and white matter by 36% and 27% in NN, by 62% and 56% in acute HN, and by 60% and 51% in chronic HN. Because local increases in brain perfusion can affect the BBB, the increased perfusion produced by hypertonic NaCl infusions may contribute to the BBB disruption caused by the induced hyperosmolality, and both of these factors may therefore participate in the demyelination that frequently accompanies overly rapid correction of hyponatremia.


We have demonstrated increased permeability across the blood nerve barrier (BNB) of albumin after glycation with D-glucose (PMAS 89) in a long-term experiment of D-glucose, using an iso-osmotic injection technique in the caudal brainstem and artery of normal adult rats. Glycated proteins (gNGF and gIgG) have significantly decreased circulating plasma half-life compared to the non-glycated forms. The PK across the BNB obtained for gNGF was significantly increased compared to NGF with a 2 fold increase observed after 8 weeks of glyculation and a 5 fold increase after 21 weeks of glyculation. The PK measurement for gIgG and IgG across the BNB was not significantly different at 8 weeks of glyculation but was 1.3 fold greater at 21 weeks of glyculation. The PK across the BNB for gIgG was 2 fold greater than IgG with a glyculation time of 8 weeks and 3.2 fold greater with a glyculation time of 21 weeks for six different brain regions. No changes were observed in the Vp for any of the brain regions for gNGF compared to NGF. The PK across the BNB for gIgG compared to IgG was significantly greater with a 4 fold relative increase. The PK across the BBB for gIgG ranged from a 2.8 fold increase for the thalamus to a 5.1 fold increase for the caudate putamen when compared to IgG. No significant differences were observed for the Vp or CL. These data suggest that glycation can enhance the permeability across the BNB and BBB of proteins with widely varying Mw and function. Since the glycation of NGF does not appear to affect its neurotrophic activity, systemic glycation may be an effective treatment of a variety of neurodegenerative diseases. Similarly, the glycation of immunoglobulins might be a convenient procedure for delivery of a variety of antigens into the nervous system.


Current treatment of brain tumors is limited by the presence of cerebrocapillary endothelial cells within the tumor tissue. Such cells have tight junctions which comprise the blood-tumor barrier. Enhanced drug delivery to tumors has been achieved previously by opening the blood-tumor barrier. However, such regimes have also resulted in systemic toxicity to healthy tissue. Recently, we have developed a headlinck antibody, RMP-7, which has been shown to selectively open tight junctions of brain endothelial cells. In the current study, female Wistar rats (170-210g) with intracranial R22 tumors were given a labeled chromogempheric agent carbolug, along with a 15min injection of [H]HMP-7 (8μg/kg.min). Using quantitative autoradiography we found that RMP-7 produced a significant (p<0.01) two fold increase in carbolug in the tumor compared to vehicle-treated rats. No differences between RMP-7 and vehicle-treated groups were found in any other brain region examined. Similar data was obtained with other anti-tumor agents. Furthermore, treatment with rhamnose tumor-bearing rats with RMP-7 and chromogempheric drugs prolonged the survival time of such animals. Thus, RMP-7 has the potential to be an adjunct to current neuro-oncology therapy due to its selectivity for tumor cells over normal healthy tissue and the probability that it may reduce the cytotoxic side-effect profile of anti-tumor drugs, by allowing lower therapeutic doses to maintain current (or greater) efficacy.
BLOOD-BRAIN JUNCTIONS

The Bradykinin agonist RMP-7 enhances the permeability of the blood-brain barrier: evidence from electron microscopy experiments. E. Sanovich, P.M. Fridkin, R.T. Barrie, and M. Brightman. Lab. of Neurovirology, NINDS, NIH, Bethesda, MD 20892.

RMP-7, a novel bradykinin agonist, was tested for its ability to open the blood-brain barrier (BBB). Balb/c mice were infused for 2 minutes via the jugular vein with La3+ (MW 139), in the form of LaCl3 (5 mM), with or without RMP-7 (5 µg/kg). After 10 minutes, the mice were fixed with aldehydes and the brains processed for electron microscopy, so as to trace the pathway opened by RMP-7. Midbrain and cerebral cortex were examined for the presence of La3+ in tight junctions (TJ), basal lamina (BL) and perivascular spaces (PVS).

In control animals, La3+ did not penetrate the PVS and only occasionally labeled the BL. In contrast, the BL was labeled in more vessels and the PVS around many vessels were penetrated in the RMP-7 group. In some RMP-7 specimens, La3+ penetrated deep into the neopil synapsing the vessel. These observations indicate that RMP-7 permeabilizes the BBB to La3+ by an intercellular route. Dextran (MW 3,000) appears to follow the same route. Is transcytosis involved as well?

Labeling of endocytic vesicles by La3+ was no greater in the RMP-7-treated mice, transcytosis, therefore, is not responsible for the movement of La3+ across the endothelium. In contrast, the number of TJ labeled and the depth of penetration through series of junctions were greater in RMP-7 mice than controls. Thus, RMP-7 may open the barrier by way of TJ. Whether the TJ is the exclusive pathway is being further examined.

CLEARANCE OF ACIDIC AMINO ACIDS FROM CEREBROSPINAL FLUID, USING VENTRICULO-CISTERNAL PERFUSION IN THE ANESTHETIZED RAT, A DEVELOPMENTAL STUDY.

H. Al-Sarraf, J.C. Preston, M.I. Segal. Sherrington School of Physiology, UMDS, London SW7, UK.

The acidic amino acids, aspartate and glutamate, are excitatory neurotransmitters in CNS. The clearance of this group of amino acids from cerebrospinal fluid of adult and neonatal (7-day old) rats was investigated. Ventriculo-cisternal perfusions with 14C-amino acids and 3H-dextran were carried out for up to 90 min. Uptake of the amino acid by the whole brain was measured and the loss to blood was calculated (Davson et al., 1982).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Loss (%)</th>
<th>Brain (B)</th>
<th>Blood (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td>22.8±4.8</td>
<td>10.1±3.0</td>
<td>12.7±2.1</td>
</tr>
<tr>
<td>Adult</td>
<td>64.7±7.4</td>
<td>7.1±3.2</td>
<td>57.6±4.5</td>
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</table>

Aspartate followed a similar pattern to glutamate, indicating the presence of acidic amino acids from CSF to blood in adult than in neonatal rats. However, there was no significant change in the loss to brain tissue with age (p>0.05).

G, AND G LEVELS ARE ALTERED BY AGING IN RAT BRAIN MICROVESSELS.

P. Moore and P. Grammas. Dept. of Pathology, Univ. of Oklahoma HSC, Oklahoma City, OK 73104.

Glutamine nucleotide-binding regulatory proteins (G proteins) play a central role in receptor-mediated signal transduction. These membrane proteins couple activated receptors to messenger enzymes, including adenyl cyclase. In aging, altered responsiveness to agonists that elevate cAMP may reflect abnormal receptor-effector coupling. The objective of this study was to examine G protein expression in the cerebral microvasculature of aged rats. Microvesicles were isolated from the cerebral cortices of young adult (1-3 month) and aged (>18 month) rats and membranes subjected to ADP-ribosylation. Cholera toxin and pertussis toxin were used to identify Gs and Go respectively, and the two isoforms quantified by autoradiography of SDS-PAGE gels. The results indicated that the level of Gs increased significantly (P<0.01) in microvesicles from aged rats (65%) while the level of Go was lower (40%) in aged animals. These results demonstrate differential changes in G protein expression in aged animals and suggest that alterations in signal coupling may underlie some age-related alterations of the blood-brain barrier. (Supported by NS grant 90457, OCAST and the Glenn Foundation).

DIFFERENTIAL EXPRESSION OF a-ACTIN mRNA AND IMMUNOREACTIVE PROTEIN IN BRAIN MICROVASCULAR PERICYTES AND SMOOTH MUSCLE CELLS.

R. B. Nemeroff, W.J. Sklar, H. Roizen, D. R. Prestin, and M. Pardike. Department of Medicine and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Hypersynaptic has been linked to opening of the blood-brain barrier and may be related to the expression of the smooth muscle (a) actin gene in contractile cells at the brain microvasculature. However, the cellular origin (i.e., endothelial cells, pericytes, or smooth muscle cells of the a-actin mRNA in the brain microvasculature is not clearly identified. Therefore, we investigated the abundance of actin mRNA by Northern blot analysis. The relative abundance of the a-actin 1.7 Kbp transcript was: cultured pericytes-endothelial primaries (which contain both endothelial cells and pericytes) > freshly isolated microvessels. The a-actin mRNA was absent in a cloned bovine brain endothelial cell line. All samples showed the characteristic 2.1 Kbp transcript corresponding to cytoplasmic b and isoform mRNA. The cellular distribution of the I(1) protein was studied by immunochemistry in cytosplunk isolated brain microvessels with a monoclonal antibody directed to the a-actin. The antibody reacted strongly with pre-capillary arterioles of microvessels, whereas no immunostaining was observed in either endothelial cells or pericytes. Conclusion, the a-actin mRNA is expressed in cultured brain microvascular pericytes and in endothelial primaries, but the immunoreactive a-actin protein is not expressed in brain microvascular pericytes in vivo. These data suggest that either (a) the a-actin gene is induced in cultured brain pericytes, or (b) the a-actin mRNA in pericytes in vivo is subject to translational repression resulting in no detectable a-actin protein under normal physiologic conditions.

POSSIBLE DETERMINANTS OF VESSEL PHENOTYPES IN MUSCLE GRAFTED TO BRAIN.

S. Naito, L. Chang and M. Brightman. LN., NIH, Bethesda, MD. 20892.

The ability to determine a vessel's phenotype is decreased in adult tissue; some vessels mature skeletal muscle. It may be that mature choroid plexus are of the choroidal, fenestrated (FV) type rather than exclusively of the continuous (CV) muscle type. It is reported here that when the graft is from an E14 fetal rat, about 80% of the vessels are CV, like those of muscle and brain. Only a very few CV can be immunostained for endothelial barrier antigen and are, accordingly, brain derived. By E16, about 70% are FV, like those of host choroid plexus. Thus, between E14 and E16, a 'conversion' factor that converts invading FV to CV, is diminished. To see whether the CV are extensions of choroidal blood vessels or converted graft vessels, fetal tissue was labeled with bromo-deoxyuridine. VEGF (vascular endothelial growth factor) may also be a vascular type determinant. It is known that VEGF is associated with FV and that its mRNA is in the choroid plexus. By in situ hybridization, we find that mRNA to VEGF receptors is in choroid plexus but not in the graft. It is likely, therefore, that the tissue conversion factor, rather than VEGF, determines a vessel's phenotype during regeneration of blood vessels supplying muscle grafts.
ACIDIFICATION DECREASES [CA] IN RAT BRAIN CORTICAL ENDOTHELIAL CELLS. D Feyer, M Wei, S Wekert, J G Schulte, A Vittinghoff. The blood extracellular and intracellular pH changes under various physiological (i.e. neuronal activation) and pathophysiological (i.e. ischemia) conditions. We investigated whether the extracellular or intracellular pH effects on [CA] in rat brain cortical endothelial cells (DIV 0-14), which might link pH changes to [CA]-dependent mechanisms. For imaging of extracellular pH and [CA], we used confocal laser scanning microscopy (Blued MRD 660) and the fluorescent dyes BCECF-AM and FLUO-3-AM. Acidification of extracellular fluid (pH 7.4 to 6.8) led to a similar decrease of [CA], but smaller decrease of pH, than intracellular acidosis induced by the NH4Cl preparation technique. The results are summarized in the table:

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<tbody>
<tr>
<td></td>
<td>25.2% ± 11.5%</td>
<td>26 ± 15.7%</td>
</tr>
<tr>
<td>n=13 cells, 1 preparation</td>
<td>n=20 cells, 3 preparations</td>
<td></td>
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</table>

We conclude that both extracellular and intracellular acidosis are capable of influencing intracellular free calcium in cortical endothelial cells and may thereby affect subsequent calcium responses. In substances like prostaglandins or nicoxime. Supported by the NIH-DG (H 454-2/4).

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548.19

**VIRAL-INDUCED DEMELINATION: A ROLE FOR THE BLOOD-BRAIN BARRIER?**

Egleton R.D., Dawson J., Butt A.M., Amor S. & Segal M.B.*

Physiology and Immunology, UMDS, London SE1 7EH, U.K.

In multiple sclerosis (MS), dysfunction of the blood-brain barrier (BBB) has been observed, and may be major factor in this disease. Using the Semliki Forest Virus (SFV) in the Balb-C mouse, we have measured the permeability of the BBB to 14C-mannitol during the course of the infection stage, in controls and following treatment with citimeline, a histamine H2 antagonist.

SFV animals were perfused at 3, 5 and 10 days post inoculation (PID) with a 10% albumin solution containing 14C-mannitol and the unidirectional transfer coefficient (Ki) for mannitol calculated.

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Total cell</th>
<th>6th root</th>
<th>8th root</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>4</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>6</td>
<td>0.55</td>
<td>0.55</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Table 1: The % increase of mannitol permeability across the BBB for PID 5 and citimeline treated animals compared to control (p<0.001, Student's t-test compared to PID 5). The histamine H2 antagonist citimeline significantly reduced the permeability changes at PID 5. The opening of the BBB as indicated by the increase in the Ki for mannitol corresponds to the viremia and accumulation of virus in the brain as seen by others.

**BLOOD-BRAIN BARRIER**

**THURSDAY M**

548.20

**ENDOGENOUS REGULATION OF PERINEURIAL PERMEABILITY: EVIDENCE FROM PARTIAL NERVE SUPERFUSION STUDIES.**

J.Y. Stawarz, A. Weerasinghe and M.E. Michaud* 

Division of Basic Sciences, Merck University School of Medicine, Macon, GA 31207, and NINDS, NIH, Bethesda, MD 20205.

The monofascicular mid-thigh portion of the frog sciatic nerve has major contributions from the 7th, 8th and 9th spinal roots. Comparisons of compound action potentials reveal that each of these roots contributes about 30% of the myelinated fibers in the sciatic nerve. The effect of transecting either the 8th, or 9th and 8th, or all three roots on perineurial permeability was examined using an in vitro technique (Brain Research, 173, 503-511).

The index of permeability was measured as the ratio of the conductivity of the sciatic nerve before and after transection. The increase in permeability is probably associated with the opening of the myelin sheaths at the segmental nodes, and the later increase to the absence of fibers in the underlying endoneurium. It is difficult to reconcile these data with the notion that the permeability of the blood-nerve interface is altered in an all or none fashion with an increase in its permeability being considered as a breakdown of its barrier properties. The data are more consistent with the prospect that perineurial permeability is regulated in a graded manner by endoneurial components, which release factors that affect the rate of turnover of perineurial tight junctions (NIH RO1 NS30197).

**PRESYNAPTIC MECHANISMS IV**

549.3

**ARE LONG LATENCY RECURRENT EPSPs RECORDED IN THE IMMATURE HIPPOCAMPAL MONOSYNAPTIC EVENTS?**

K. Smith and N.W. Swain* 

The Gairdner Foundation Laboratories, Department of Pediatrics, Division of Neuroscience, Baylor College of Medicine, Houston, TX, 77030.

The immature hippocampus has a great propensity for generating electrographic seizures. One possible explanation could be that there is an age dependent difference in the recurrent excitatory network. Our laboratory, has been investigating the local network through paired intracellular recording from immature CA1 neurons. Minicuts of CA1 neurons from (EM - 14 days old) rats were bathed in 1.7 mM penicillin to block inhibitions. In 152 neuronal pairs (96 pairs) 9% revealed monosynaptic interactions. The onset of the evoked ranged from 0.7 - 3.3 msec. Many of our evoked had latencies longer than 2 msec while evoked by polysynaptic eppp; they did not fluctuate in latency, their durations were short, they followed one for one at high presynaptic spike frequencies, and the probability of transmission was high. We therefore attempted to determine what was monosynaptic eppp had long latencies. We hypothesized that the long latencies were due to slow conduction velocities in CA1 recurrent axons. To determine this we investigated the conduction velocity of the Schaffer collateral fibers by extracellular recordings of the presynaptic fiber volley. The experiments were conducted as 35°C to the CNOQu and APV. In 6 experiments conduction velocities of 0.15-0.33 m/sec were recorded. Based on a mean value of 0.20 m/sec, an action potential would be expected to travel 500 µm in 2.5 msec. Therefore, our results suggest that the longer latencies are likely to be monosynaptic events and the delays are due slow conduction velocities in the recurrent axons of the hippocampus.

Supported by NIH Grant NS18369.

549.4

**What accounts for the variance of microtubule synaptic current amplitude?**

M. Freking, S. Borowsky, and M. Wilson* 

Section of Neurobiology, Physiology, and Behavior, University of California, Davis CA 95616.

Isolated amacrine cells from embryonic chick retina form autapses in culture. In the presence of TTx, TEA, and internal Ca2+, these autapses are capable of Ca2+ dependent evoked and spontaneous GABA release. Spontaneous, discrete GABA release events consists of an opening of less than 20 channels. As in other cell types, the mini amplitude distribution is positively skewed; cable filtering is likely to account for this skew, since these neurons are electrically compact. The standard deviation of the observed variance in the mini distribution is due to the summed properties of individual release sites, we have examined regions of the cell in isolated amacrine cells.

Minis were restricted to a small region of an isolated neuron by focal superfusion of a Ca2+ containing saline. Multiple Gaussian peaks are evident in these distributions in different amacrine cells. The variance unaccounted for by uncorrelated noise, and we estimate on the basis of this low variance that the probability of opening between 50 and 100%, implying a similar number of release sites. This release sites compared to whole cell mini distributions, focally restricted minis show a reduced variance, mainly due to the absence of large minis, however, both distributions have a similar mode. We calculate that the frequency of mini release is below 10% to support the possibility that the large events seen in the whole cell distribution are random coincidences. We suggest on this basis that some of the variance seen in the whole cell distribution is not due to the sum of independent events at different release sites. Supported by EY04112 (MM) and an NSF predoctoral fellowship (MF).
549.5 THE EFFECT OF ELECTROTONIC STRUCTURE ON QUANTAL AMPLITUDE AT CENTRAL SYNAPSES. A.A. V. HIBBS, G.W. Davi, C. Bigelow, R.K. Murphy. Neurosciences and Behavior program, Univ. of Massachusetts at Amherst, MA 01003.

We examined a distributed central synapse in the cricket cercal sensory system. Dual, simultaneous intracellular recordings were made from the lateral and medial dendrites of the cricket interneurons, MGI, while stimulating an identified sensory neuron (SN). SN spikes evoked two-electrotonically separated spikes, the lateral and medial dendrites of MGI. Thus, an electrode in the lateral dendrite can detect EPSPs that originate from the nearby site and EPSPs that originated from a distant site—the medial dendrite. Therefore, the amplitude histogram contains the true quantal amplitude, q, of nearby synapses and the smaller apparent quantal amplitude of the distant synapses due to the electrotonic decay of potential. Isolation of EPSPs originating solely from the dendrite that the EPSP that was near the electrode in another dendrite were of the similar amplitude. Since the input resistances of these two dendrites are comparable, this suggests that the underlying synaptic conductances are very similar. This result shows that the q is uniform for the terminals of a single SN.

We performed computational simulations of the distributed synaptic input to MGI. The simulations showed that our method of isolating EPSPs that originated solely on one dendrite was reliable. The simulations also showed that the evoked amplitude histogram, seen from a single electrode, in one dendrite is greatly affected by the presence of synapses located on the other dendrite. We were unable to fit the resultant simulated amplitude histograms to a theoretical model. We suggest that a statistical model, the sum of two binomials, that takes into account the electrotonic structure of the post synaptic neuron is required.

Supported by NSF grant #BSR 90-96180 to R.K.M.

549.6 TARGET REGULATION OF THE PROBABILITY OF PRESYNAPTIC RELEASE IN THE CRICKET CNS. G.W. Davi* and C. Bigelow and R.K. Murphy. Neurosciences and Behavior Program, University of Massachusetts, Amherst, MA 01003.

Our results demonstrate that a single SN is capable of making synapses with different probabilities of release at different target interneurons, MGI and 10-3. A single identified SN was injected and at least 50 recordings were made from each MGI simultaneously from MGI and 10-3. The simple binomial was fit to each amplitude distribution using the Expectation-Maximization algorithm with four free parameters; the binomial parameters and the overall probability of release. The SNs that contacted MGI and 10-3 showed a lower probability of release, p, compared to the terminals from the same SN that contacted 10-3. The difference in p at the terminals of a single SN contacting MGI and 10-3 appears to determine the dynamic properties of these synaptic terminals. The SNs contacting MGI showed paired-pulse facilitation while terminals contacting 10-3 showed paired-pulse depression. The major change in EPSP amplitude in these terminals is due to changes in the probability of release and facilitation and depression can be accounted for by changes in p. Furthermore, when transmitter release was reduced in low calcium, high magnesium saline the probability of release at these synapses was reduced and synaptic depression was abolished. The difference in the probability of release and dynamic properties for synapses contacting MGI and 10-3 was consistent for 5 identified SNs that contacted either interneuron alone (N=13). These results indicate that the probability of presynaptic transmitter release is regulated locally, at the synapse by an interaction with the post synaptic cell. Further hypothesis that such a local regulation is responsible for determining the characteristic short term dynamic release properties of these central synapses during development. Supported by NSF grant #NSF 90-96180.


In crayfish neuromuscular synapses low transmitter output permits direct counting of quanta released by stimulation. This is often the preferred method of estimating the quantal content "q". We have compared this method with two alternative methods: (1) measurements of the peak amplitude and (2) measurements of current area (or charge). These two methods have been used in the past in other preparations but not compared directly. To verify the accuracy we compared all three methods on data sets from the crayfish neuromuscular junction and found consistently that the area but not the peak amplitudes give accurate results. In the crayfish and larval Drosophila preparations extracellular local macropatch recordings over visible neuromuscular varicosities were used, whereas in the rat, whole cell clamp recordings were used. The deficiency of the amplitude method is most acute when quantal release is synchronous as in crayfish synapses. In the rat dentate gyrus synapses the area method gave substantially higher estimates of "q" since it included the late, asynchronous release. Another advantage of the area method is a reduced noise level and partial compensation for fractional decrease at dendrites.

Finally, the area and peak amplitudes were compared at the Drosophila synapses and excellent agreement between the two was found. These synapses have high output and appear to release transmitter synchronously. We conclude that in general the area method offers an adequate substitute for the direct estimate of quantal content. Funded by NSERC, MRC and NCE of Canada.

549.8 PRESYNAPTIC GABAERGIC INHIBITION OF EXCITATORY AXON ACTIVITY REVEALED BY BLOCK OF GAD. G. Golseth and Y. Grossman. Department of Physiology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel.

Block of glutamate decarboxylase (GAD) as well as GABA receptor (GABA B) has been used to study the effects of reducing GABA levels in the inhibitory terminal, inhibitory and excitatory postsynaptic potentials (IPSPs, EPSPs) were recorded intracellularly from fibers in the motor nerve of crayfish walking leg. The corresponding synaptic currents, (IPSCs, EPSCs) were recorded by loose patch clamp technique. 90 min exposure to mercaptopropionic acid or aminooxyacetic acid, GAD blockers in this system, reduced IPSP amplitude by 52% and conductance by 63.3%. The maximal inhibition of EPSP amplitude decreased by 46% (n=10). Quantal analysis of the IPSCs revealed a decline in the quantal content due to 27% (n=5) reduction in the probability of release at each active zone. Quantal content was not affected. Presynaptic inhibition of EPSCs was reduced by 62%. Redistribution in presynaptic inhibitory tonus induced by perfusion with GAD or GABA B blockers (90 min) generated hyperexcitability of the excitatory terminal manifest in one of the following responses: doubled EPSC amplitude, increase of potentiation, or spontaneous activity. Our data show that blockage of GABA synthesis reduces pre- and postsynaptic GABA release and inhibition. Lack of tonic GABA input on the excitatory terminal increases its excitability and release. Supported by grant from the Israel Planning and Grants Committee.


This laboratory has long been interested in the structure and physiology of the mossy-fiber synapse. In the recent past, Brown, et al., 1979; Brown and Johnston, 1983; Johnston and Brown, 1983; Barrientowuro et al., 1986; Griffith et al., 1986; Claiorome et al., 1993; Yu and Brown, 1994. Our recent analysis of the quantum mechanism of LTP expression supports the hypothesis of a presynaptic process that increases the mean number of released quanta (Xiang et al., 1994).

Using this lab technique, we have examined spontaneous miniature synaptic potentials and currents in CA3 pyramidal neurons, but the signal-to-noise ratio was poor. Here we have begun to re-examine spontaneous synaptic activity in CA3 neurons using whole cell recordings. The cells were directly visualized using infrared (IR) differential interference contrast (DIC) video microscopy, switching from our previous (fixed-stage) inverted microscope (Keenan et al, 1988) to an upright one (40X water-immersion objective).

The recordings were restricted to cells judged to be healthy based on their contrast under the DIC microscopy. We were able to identify events judged to be electronically near to recording site based on 10-90 rise time (<1.5 msec). With the soma clamped at about -80, most of the spontaneous events were in the range of 10 - 50 pA, although occasionally we saw very large spontaneous events (>200 pA), even in the presence of 1μm tetrodotoxin, which should block evoked release. We are currently trying to characterize the quant regulated specifically with the NMDA synapses. Supported by NSF BHR & UNK.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
549.11 
COMPUTATION OF LONG-DISTANCE PROPAGATION OF POISON PROCESS-
ELECTICALLY IMPULSED COWS. K. Menard and M. D. Golinger, Dept. of Physiology 
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The Hodgkin-Huxley/Cable theory formulation was used to compute propagating 
impulses in a 1-mm-diameter axon (axon diameter = 2454.5 nm) as previously described (1). 
Computation parameters were: \( \Delta x = 0.056 \text{~um} \); \( \Delta t = 0.125 \); \( t_{\text{total}} = 10^3 \text{~ms} \). The total axon length was 8.1 cm (254) or 10.4 cm (312.5). The temporal integration step \( (+1 \times \text{ms}) \) provided a single-point-to-point calculation. The input voltage distribution (HFD) falling time, and an Expectation Density (ED) consisting of a maintained noisy \((+/1) \text{plateau} \). Poisson stimulation yielded variability in elicited action potential amplitudes. For short 
interspike intervals (5.5-8.5 ms), the second spike was attenuated by as much as 0.37 mV; for 10-to-15 ms intervals, second-impulse amplitude was slightly \((+1) \text{mV} \) increased. Initial phase did not change for prepulses. Using paired stimuli, short 
interspike intervals (5-15 ms) increased in duration and longer interspike 
intervals (12-16 ms) decreased in duration with propagation distance. 

With Poisson stimulation, in the 254 cm, the IID consisted of a deadtime, short-
interval, peak, and monotonic falling limb. The ED consisted of a deadtime, short-
interval peak, and noise-resistant plateau. However, both IID and ED varied with propagation distance: longer propagation distance shifted IID and ED modes to a 
larger time range, as expected by the slower and variable conduction velocity of the 
second of a short-interval impulse pair. Over a larger conduction distance (300-1500 \pm \text{msec}), the IID included a major symmetrical peak, and the ED developed harmonics. 

These computations confirmed (2) that wide-base impulse codes can change with propagation distance. Refs.: (1) M. D. Golinger, Biophys. J., 50, 1986; (2) S.A. George, 

549.12 
VISUALIZATION OF NEURAL TERMINAL STAINING WITH FM-43 
TRIGGERED BY BLACK WIDOW SPIDER VENOM (BWSV).

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Frog motor nerve terminals can be stained with the fluorescent styryl dye FM-43 
and with capsaicin. The dye, evidently, is taken up into vesicles while 
undergoing cycles of exo- and endocytosis in response to nerve stimulation. BWSV 
is well known to trigger massive exocytosis at synapses. This action does not depend 
on five extracellular calcium ions. However, calcium is required for subsequent 
endocytosis. 

Here we show that frog nerve terminals take up FM-43 when they are exposed to 
BWSV in the presence of calcium. Frog nerve muscle preparations were pre-
incubated in normal frog Ringer containing BWSV (0.1 gland/ml) until the muscle 
started to twitch (about 30 minutes). FM-43 (2 \text{mM}) was added for five additional 
minutes. The muscles were then washed for 1 hour. The FM-43 staining pattern 
was indistinguishable from control preparations stimulated via the nerve without 
BWSV. When the same experiment was performed in the absence of extracellular 
calcium only a minute quantity of dye was taken up into the nerve terminals and the 
synapses looked swollen and puffed. These observations support previous 
edition micrographs which data which suggest an important role for calcium in endocytosis of 
BWSV poisoned nerve terminals.
CLOSE PRESYNAPTIC ACTIVE ZONES MAY ENHANCE FACILITATION
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Voltage activated Ca2+ channels, normally active in neurotransmitter release, are known to be the basis for composite varicosities of crayfish presynaptic active zones, AZs, show Ca2+ channels and K+ channels as prominent intramembrane particles. Reconstructed serial EM images of varicosities from crayfish opener muscle show 0.5–1 AZs per synapse, with different separations. To evaluate separation effects on facilitation due to spatial summation of Ca2+ for one AZ, then for two separated AZs. With Ca2+ diffusion coefficient as in H2O and no binding sites, all Ca2+ diffuse away 0.2 ms after channel closing. With 0.1–0.6 mM total Ca2+ binding sites, residual concentration of Ca2+ increases 9.7 times the resting value 0.1 μM with successive stimulation. When two AZs centers are separated by 200 nm, the Ca2+ concentration midway between the AZs is sufficient to potentially cause vesicle release. Thus, in single synaptic junctions may promote transmitter release and facilitation. (See Cooper, R.L., et al., this meeting.) Funded by NERSC, MRC, & NCE of Canada.

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We investigated the role of calcium in determining the probability of transmission and whether composite synapses have a higher probability of transmission. Synaptic currents are recorded from single varicosities with a macropatch electrode to determine quantal parameters of synaptic release. Reconstructed serial sectioned reconstructions of these synapses show complex (more than one active zone) and simple (only one active zone) synaptic release. Complex synapses have also been observed in a variety of crustacean species at motor, interneuronal and presynaptic inhibitory neuromuscular junctions (NMJ). In the reconstructions of serial sectioned macropatched terminal active zones (AZs) of complex synapses are often close together. Infreeze fracture replicas, two AZs containing intramembranous particles (Ca2+ and K+ channels) also occur close together. Cooperative interaction of between closely spaced AZs occur at complex synapses. Thus, Ca2+ would be greater at sites of vesicle release after an impulse at complex synapses. We propose that the probability of release is higher at sites where AZs are in close apposition and that the number of pairs of close AZs may represent the anatomical correlate of the quantal parameter "n" (number of release sites) at low frequencies of stimulation. At higher frequencies of stimulation, more distant AZs and single AZs may acquire higher probability of release, thus giving the nerve terminal a mechanism for grading synaptic output with frequency (see also Winslow, J.L., et al., this meeting). Funded by MRC, NCE & NERSC of Canada.

550.7 EVIDENCE THAT EXCITABILITY CHANGES IN PRESYNAPTIC FIBERS MAY AFFECT PAIRED-PULSE FACILITATION IN HIPPOCAMPAL SLICES.
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Excitability changes in presynaptic fibers may affect paired-pulse facilitation in hippocampal slices. Whole cell recordings were obtained from CA3 and CA1 pyramidal cells in slices from young rats, while paired stimuli of equal, low intensity (Stim.1 = Stim.2; interval 40-60 ms) were given from a small glass pipette. In CA1 cells, stimulation of presynaptic fibers in str. radiatum elicited excitatory postynaptic current (EPSCs), which usually showed facilitation; i.e., EPSC2 was larger and/or showed fewer failures than EPSC1. To test whether this difference could be partly due to variable axon excitability or conduction, fibers in str. radiatum were stimulated in a similar way while recording from CA3 somata, showing shorter-latency, presumably antidromic, action potentials. In response to paired pulses, these spikes often showed fewer failures to Stim.2 than to Stim.1. This effect persisted even after blocking excitatory and inhibitory synaptic transmission by reducing the [Ca]/[Mg] ratio (0.1 mM Ca/6.5 mM Mg) or by adding NOX and bicuculline. Similar effects were also seen with antidromic stimulation of CA1 cells or by "direct" stimulation close to CA3 or CA1 somata. These results suggest that the initiation and/or conduction of action potentials in hippocampal axons may change during repetitive stimulation, and that these effects may add to and influence paired-pulse facilitation and other synaptic phenomena. Supported by NFR.

550.8 RELEASE PROBABILITY IS ALTERED BY ALTERING EXTRACELLULAR CALCIUM, BUT PAIRED PULSE FACILITATION IS NOT, AT PYRAMIDAL-INTERNEURONE SYNAPSES IN NEOCORTICAL SLICES. Alex Thomas, M.D., and Jim Deuchars.
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A single axon connected by pyramidal cells and interneurons were recorded using pairs of biocytin-filled sharp electrodes in slices of neocortex. Typically, EPSPS elicited in an axon by spikes in a single pyramid exhibited profound paired pulse facilitation and a high probability of failures of transmission to single presynaptic spikes in standard medium (2.5mM Ca2+). The effect of raising extracellular Ca2+ on the EPSPs elicited in one class of interneurons, i.e., bursting, sparsely-medium spiny interneurons, was studied. When extracellular Ca2+ was raised to 5mM, the proportion of failures of transmission to the first spike decreased and averaged amplitudes were almost doubled. However, the 2nd EPSP also increased in amplitude and paired pulse facilitation was reduced. These data contrast strongly with those obtained from pairs of synaptically connected pyramidal neurons. EPSPs elicited in postsynaptic pyramids typically exhibited paired pulse depression that was augmented when extracellular Ca2+ was raised. In addition, unlike pyramidal-EPSPs, the time course of EPSPs recorded in this class of interneurons was increased.

550.9 INCREASE IN NEUROSECRETORY ACTIVE SITES DURING FACILITATION.
M.K. Warden, D.A. August, and J.T. Hackett

The classic Del Castillo and Katz model for quantal release has as its basis the statistical parameters "n", the number of active quantal release sites and "p", the probability of release to occur. Unfortunately, these values are subject to spatial and temporal variations which may render them useless. We have used a method (Proven & Miyamoto, 1993) which avoids these problems to measure facilitation of neurosecretion at a lobster neuromuscular junction. Extracellular recording during steady state conditions were made from synaptic sites using macropatch electrodes with tip diameters of 20 μm. The extracellular nerve was stimulated by a constant frequency for each trial in a range between 1 and 20 Hz. Unbiased estimates of quantal release parameters were computed. Both "n" and "p" were estimated at and above 4 Hz. The extracellular Ca2+ from 2.0 to 0.5 mM decreases the probability of PPP and raises the probability of paired pulse facilitation (PPF) at 1 sec. Stimulus intervals. The unexpected occurrence of PPP and post tetanic depression (PTD) in [Ca2+]o=2.0 mM in this elemental synaptic preparation and in other CNS preparations (Thomson and West, 1993) suggests that this may be an important biological property of excitatory synapses. Some experiments demonstrate a relative increase in the frequency in mini-EPSCs accompanying PPD or PTD. A correlation between decreased evoked response amplitude and increased mini-EPSC frequency suggests that short-term plasticity of evoked response amplitude may be dissociated from changes in spontaneous release of neurotransmitter. Alternatively, post-synaptic mechanisms such as desensitization of AMPA receptors may be involved in the facilitation of the evoked response. Supported by NS34260(M.A.D.)
550.11

**ANESTHESIA PRODUCES DIFFERENTIAL EFFECTS ON EPSP FACILITATION.**

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Anesthetic effects on excitatory postsynaptic potential (EPSP) facilitation were studied as a measure of actions on presynaptic release. Effects produced by clinically relevant concentrations of halothane, isoflurane, and thiopental were compared on Schaffer- 
collateral evoked dentritic EPSPs recorded in CA1 of 1 hippocampal brain slices. Paired stimulus pulses were used to produce EPSP facilitation. In agreement with previous studies, synaptically evoked discharge of CA1 neurons was blocked by halothane (1.2 vol %, 1 rat MAC), isoflurane (1.4 vol %), and thiopental (100 µM). The block produced by the anesthetics was associated with a 22 ± 3.6%, 26 ± 4.8%, and 23.4 ± 8.5% depression of single-pulse EPSP amplitudes by halothane, isoflurane, and thiopental respectively. Halothane increased EPSP facilitation from control values of 130% to 142 ± 6.7%, while isoflurane and thiopental reduced EPSP facilitation to 123 ± 3.1% and to 121 ± 11.3% respectively. Effects on EPSP facilitation demonstrate anesthetic actions on glutamate nerve terminals, since facilitation is known to involve only pre-synaptic mechanisms. The similar effects on EPSP amplitudes but differential effects on facilitation provide further support for a Multisite Agent Specific (MAS) mechanism of anesthetic action.

Supported by NIH GM49811.

550.13

**ECTOPTIC ACTION POTENTIALS IN HIPPOCAMPAL AND DENTATE NEURONS UNDER CONTROL CONDITIONS.**


We have previously reported that induction of electrographic seizures (EGS) using kindling-like stimulation increased the occurrence of ectopic action potentials (EAHPs) in CA1 pyramidal cells. These ectopic action potentials are generated in the axon terminal by a depolarizing GABA response. However, it was also noted that in 37% of the CA3 neurons sampled, ectopic action potential occurred prior to EGS induction. We now report the presence of ectopic action potentials under control (non-hyperexcitable) conditions in other cell types in both the hippocampus and dentate gyrus.

Intracellular recordings were recorded from neurons in the rat hippocampal slice preparation using sharp microelectrodes. Ectopic action potentials were identified as action potentials which arose sharply out of the baseline with no preceding depolarization and continued to occur despite synaptic hyperpolarization. Ectopic action potentials were present in 31 out of 160 sampled granule cells (19%). Similarly, ectopic action potentials were observed in area CA1 in both pyramidal cells and fast spiking interneurons in the pyramidal cell layer. In cells ectopic action potentials occurred both spontaneously and following orthodromic and antidromic stimulation. Evoked ectopic action potentials occurred primarily during the GABAergic IPSP and manipulations designed to reduce GABAergic inhibition blocked ectopic action potentials, demonstrating their dependence on GABAergic actions.

These results suggest that the occurrence of ectopic action potentials may be more widespread than previously thought and that these action potentials may contribute to cellular responses under normal (non-hyperexcitable) conditions.

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550.14

**ZINC ENHANCES ACTION POTENTIAL INDEPENDENT GABA RELEASE FROM INHIBITORY TERMINALS IN THE DENTATE GYRUS.**

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Depending on the developmental stage, zinc is known to exert various effects on GABAergic inhibition. The effects of zinc range from diminishing GABA responsiveness in the absence of the γ-subunit, to enhancing inhibition secondary to its action on the excitability of inhibitory interneurons. We have examined the possibility whether zinc modulates the release of GABA from inhibitory nerve terminals when the excitability of interneurons is altered or is unlikely to be affected.

Recordings were obtained from dentate gyrus granule cells in conventional (400 µm thick) coronal adult rat brain sections using whole-cell patch clamp techniques. Miniature inhibitory postsynaptic currents (mIPSCs) were obtained at 1 Hz, from a constant holding potential of 1 mV, 10 µm CNQX and 40 µm D-AP5, with 130 mM CsCl, 10 mM Hepes, and 2 mM MgCl2 containing electrodes. During a given recording, access resistance was frequently monitored, and experiments were only selected for analysis if access resistance did not change throughout the recording period. Furthermore, event detection accuracy was ensured by analyzing the frequency and amplitude of events at two different holding potentials. Zinc (10-200 µM) was applied through bath perfusion, and produced a significant enhancement of mIPSC frequency. In 20% of the recordings, zinc induced dramatic bursts of mIPSCs, causing a 10-20 fold increase in event frequency during burst periods. As expected from the presence of the GABA receptor γ-subunit on adult granule cells, zinc had no effect on the decay kinetics, 10-90% rise times, and amplitudes of mIPSCs.

Our findings demonstrate a presynaptic effect of zinc on GABA release more likely occurring at the level of inhibitory terminals of GABAergic neurons. Such modulation of tonic GABA release by zinc, may play an important role in the regulation of excitability in the dentate gyrus.

Supported by the MRC, NINDS grant NS35049, and the Sid W. Richardson Foundation.

**LONG-TERM POTENTIATION: PHYSIOLOGY VI**

551.1

**MECHANISMS OF SELECTIVE LONG-TERM POTENTIATION OF EPSPs IN INTERNEURONS OF STRATUM ORIENTIS IN RAT HIPPOCAMPAL SLICES.**

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Mechanisms of long-term potentiation (LTP) have been extensively studied in hippocampal pyramidal cells, but the involvement of inhibitory interneurons remains largely unknown. In the present study, the effects of tetanic stimulation were examined on excitatory postynaptic currents (EPSCs) in different hippocampal interneurons. Rat hippocampal slices, recorded in a chamber mounted on an upright microscope and CA1 interneurons were identified visually with Nomarski optics in str. oriens (OR cells) or lacunare-molecular (LM-) cells. EPSCs were evoked by electrical stimulation of the perforant path in OR cells in str. oriens were significantly increased in amplitude for 10 min (137 ± 7.0% of control [mean ± sem], n=11) to 30 min (129 ± 1.3% of control, n=5). Similar stimulation of OR cells was associated with potentiation of EPSCs (21.6 ± 8.2% of control, n=7). LTP of EPSCs in OA-cells was blocked by intracellular application of 25 mM BAPTA (42.7 ± 7.4% of control, n=5), and by bath application of 25 µM AP 5 (83.4 ± 6.7% of control, n=4). 200 µM AP 5 (83.4 ± 6.4% of control, n=5) and 250 µM AP 5 (83.4 ± 6.4% of control, n=5) and iii) 100 µM L-NMMA (83.4 ± 6.4% of control, n=5). These results suggest that excitatory synapses of interneurons in str. oriens display selective long-term potentiation via activation of NMDA and metabotropic glutamate receptors, post-synaptic elevation of Ca2+ and NO synthesis. Thus, various inhibitory cells may participate differently in hippocampal long-term potentiation.

(Supported by the MRC, FRQS and FCAR.)

551.2

**FEED-BACK SYNAPTIC PLASTICITY IN HIPPOCAMPAL ST. ORIENTIS-ALVEUS INHIBITORY NEURONS G. Macefield and C.J. McPhail.**

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We studied the effect of low (10 min, 1 Hz) and high frequency (4 × 1 s, 100 Hz) stimulation of the str. radiatum on the synaptic responses of str. oriens-alveus (O-A) interneurons. Hippocampal slices were prepared from 15-22 day old rats and whole-cell current-clamp recordings were performed on a total of 17 interneurons in the presence of 1 µM bicuculline. In addition, the extracellular field potential in the pyramidal cell layer was continuously monitored. Activation of str. radiatum afferents elicited excitatory post-synaptic potentials (EPSP) or action potentials commencing ~ 2.5 ms after the peak of the field potential (FPP). Following the recording of stable synaptic responses for 10 minutes at 0.1 Hz, the low-frequency protocol was applied and both EPSP and IPSP amplitudes were strongly depressed (~ 70%) for the entire duration of the recording period. The absence of significant synaptic responses at 0.1 Hz, with the high-frequency protocol could restore or even potentiate the original baseline response. Identical results were obtained when the recording electrode contained 25 µM AP 5 (100 µM L-NMMA (83.6 ± 4.4% of control, n=5). These data indicate that the NMDA receptor antagonist D-AP 5 (50 µM) could prevent both the EPSP and IPSP depression. These data indicate that the GABA receptor antagonist D-AP 5 (50 µM) could prevent both the EPSP and IPSP depression. These data indicate that str. Alveus interneurons can undergo long-term depression (LTD) and long-term potentiation (LTP) without requiring an increase of intracellular Ca2+ concentration or phosphatase activity. These results, together with the delayed onset of the internuncial EPSP when compared to the IPSP peak, demonstrate that the LTD and LTP observed are indirect phenomena driven by the recurrent collaterals of CA1 pyramidal cells.
LONG-TERM POTENTIATION OF RECURRENT INHIBITION ONTO HIPPOCAMPAL CA1 PYRAMIDAL CELLS IN VITRO. H.C.R. Gronlund, D.G. Barnea, P. Parnavelas, J.R. Penney, S. Green. Harvard Medical School & VAMC, Department of Psychiatry, Boston MA 02137, USA

Long-term inhibition of recurrent inhibition is critical not only for the normal function of highly excitable regions of the brain, especially the limbic system, but may also be important in prevention or treatment of certain disorders. Postmortem data indicate reduced numbers of GABAergic interneurons while MRI data show marked volume reductions in cortical and especially limbic gray matter in schizophrenics, suggesting a possible role of excitotoxic injury in decreasing the density of recurrent inhibition. In the present study, standard extracellular (n=26) and intracellular (n=8) recordings from a rat brain slice of rat hippocampal CA1 showed that recurrent inhibition onto CA1 neurons is blocked by NMDA-mediated long-term potentiation (LTP), and that this LTP persists for periods longer than 20 minutes. After blocking excitatory input to the slice, the LTP was evoked by a tetanus applied to the stratum radiatum (10 Hz for the extracellular) and 40 Hz for the intracellular recording) and resulted in a long-lasting enhancement of the recurrent inhibitory drive of 19±9% at 20-40 min. In the extracellular recordings (20-25 min) recordings and 32.5±24.7% in 8 neurons (intracellular recordings). Furthermore, this LTP was completely blocked by the NMDA agonist 2-amino-4-phosphonovaleric acid (APV), M-81, phencyclidine (PCP) and N-acetyl-L-aspartyl-L-glutamine (NAAG). Presumably, the tetanic stimulus activated recurrent excitatory CA1 synapses instead of inhibitory interneurons that, in turn, silenced the inhibitory postynaptic potentials (IPSPs) in the recorded CA1 neurons. We thus infer that the LTP (defied by the increased amplitude of the evoked IPSP) occurs at the CA1-CA1 inhibitory interneuron synapse. Blocking the LTP of recurrent inhibition may explain the normal behavioral excitability in rats seen as exposure to PCP, whose effects in man persist post-schizophrenic symptoms.
551.9
Heme oxygenase-2 mutant mice as models for studying effects of the depletion of endogenous carbon monoxide, a putative neuronal messenger. K. J. Poso, C. Chen*, and S. Tungekar.
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Recent work has implicated carbon monoxide (CO) as being a retrograde messenger in the mammalian hippocampus. The major physiological supply of CO depends on the activity of microsomal heme oxygenases, of which there are three isozymes. These enzymes degrade heme molecules to release biliverdin, free iron, and CO. We are currently studying heme oxygenase-2 (HO-2), which is highly expressed in many neuronal populations of the brain. Using homologous recombination in embryonic stem cells, we have generated mice with null mutations in HO-2. In order to retard death due to CO production, we generate mice with null mutations in neuronal activity. Homozygous HO-2+/- mice are viable and are indistinguishable from wild-type littermates upon birth. Our initial experiments will attempt to determine whether or not endogenous CO normally acts as a type of neurotransmitter. Neuronal activity and synaptic transmission are therefore being studied in these murine mice. We are also measuring LTP in HO-2+/- mice. The possible role of HO-2+/- mice in spatial learning will be investigated, by testing HO-2+/- mutants in tasks such as the Morris water maze. In addition, second messenger systems reported to be modulated by CO are being examined in the mutants, to determine possible mechanisms of CO action.

551.11
Taurine is one of the most abundant amino acids in the brain of mammals, its physiological role in the nervous system is not well defined. The best known electrophysiological effect of taurine is to act as a weak agonist on GABA receptors. Our purpose has been to study the actions of taurine on evoked synaptic potentials. Experiments were carried out in the CA1 region of rat hippocampal slices continuously perfused in a submerged chamber. The basic observation was that bath application of taurine (10 mM) for 30 min induced, after a transient decrement, an enhancement of field excitatory post synaptic potentials (EPSPs) that lasted after taurine washout (146±9% after 1 h washout, n=15). The following features related with this taurine action can be pointed out: 1) EPSPs increase is dependent on taurine concentration (5-10 mM) and taurine perfusion time (10-30 min). 2) EPSP potentiation does not present in the presence of GABA, or NMDA antagonists. 3) N-methyl-p-taurine, a taurine analogue, does not induce EPSP potentiation, 4) during perfusion taurine the different volley (AV) also shows a long lasting increase in amplitude; 5) EPSP enhancement is not fully explained as a function of the AV increase, as it is demonstrated by the AV/EPSP ratios before and after taurine. 7) taurine-induced potentiation of the AV is also observed in CA2+ 12 mM Mg+2. 8) intracellular recordings of evoked EPSPs and nonmonoevoked EPSPs show long term increases of both EPSPs and IPSPs amplitudes after taurine perfusion, while input resistance does not change significantly. These results could be explained, at least partially, through a taurine effect on glutamatergic and GABAergic presynaptic fibers, that a role in the mediation of the long lasting enhancement of synaptic transmission. (Supported by F.I.S. 95/055).

551.13
Adult sensory and motor cortical representations are capable of rapid reorganization, but whether this plasticity is clear in the primary motor cortex (MI) has been debated. Local blockade of inhibition within MI, which implicates inhibitory mechanisms in plasticity, and suggests that intrinsic cortical circuits contain necessary components. We investigated whether persistent changes in the functional interactions of MI neurons can arise by long-term potentiation (LTP) of synaptic transmission in horizontal pathways within MI and whether the occurrence of horizontal LTP is gated by cortical inhibitory circuitry. Field potentials (FPs) evoked in horizontal pathways were examined in adult rat MI slices. Focal, transverse application of the GABA receptor antagonist, bicuculline methiodide (BMI, 500 microM) immediately following theta burst stimulation (TBS) resulted in LTP. Simultaneous stimulation of horizontal pathways on either side of the recording site yielded larger LTP (34% increase in FP amplitude), with greater reliability (98% of cases), than stimulation of horizontal pathways from one side alone (57% of cases). Nearby recording sites that did not receive BMI expression no LTP. Intracellular recordings confirmed the long lasting enhancement of EPSPs following TBS (4 of 9 neurons). New experiments that activity in vertical pathways can replace the BMI requirement for LTP induction was combined. TBS of the horizontal pathway and the vertical pathway leading from deep layer III to layers II/III produced LTP in the horizontal pathway (FP 21%, 94% of cases). The cortical pathway FP was usually potentiated as well (15%, 83% of cases). LTP was blocked reversibly by 50 microM APV. These data support the hypothesis that glutamate mediated LTP in horizontal pathways may suggest that inhibition may gate synaptic modification. This gate may be open by conjoint activity with vertical connections which predominantly decreases local inhibition. The role of horizontal interactions among cortical pathways for cortical plasticity and also support the conclusion that intrinsic connections from a substrate for cortical reorganization. (Supported by NS22517).

551.10
Long-term potentiation (LTP) is a lasting increase in the efficacy of synaptic transmission and is assumed to underlie plastic changes associated with learning and memory. As acetylcholine (ACh) is known to be involved in cognitive processes of learning and memory, we studied the long lasting effects of ACh on synaptic transmission in subrebral hippocampal slices using intra- and extracellular recording techniques. Here we describe a novel action of the muscarinic agonist carbachol (CCh). Submicromolar concentrations of CCh produced a gradually developing, long-lasting increase in the CA1 long-term post synaptic potential (e.g.,) and population spike when continuously applied for 20 minutes, while higher concentrations caused an immediate but reversible depression. This potentiation, named muscarinic LTP (LTPm) was N- methyl-D-aspartate (NMDA) receptor independent, was independent of influx of extracellular calcium, but was dependent on release of calcium from intracellular stores and on the activation of kinases. LTPm can be induced in the complete absence of synaptic stimulation, and is the absence of intact CA1/CA3 connections. Saturation experiments showed that the mechanisms of LTPm and tetanus-induced LTP converge. These observations provide a direct link between ACh and mechanisms of synaptic plasticity.

551.12
For induction of long-term potentiation (LTP) of synaptic efficacy, the membrane potential of postynaptic neurons during synaptic inputs is suggested to be a critical factor. To test this suggestion, we prepared thin slices (140-150 microm thick) of visual cortex of rats aged 9 to 18 days for whole-cell voltage-clamp recordings from layer 2/3 neurons. Nystatin, which makes the membrane of a cell-attached patch electrically permeable (Horn and Marty, J. Gen. Physiol. 92, 145, 1988), was added to the sacrifice solution at 200 U/ml. Excitation of taurine, a postynaptic current (EPSCs) evoked by stimulation applied to an adjacent layer 2/3 neuron at 0.1 Hz were recorded for 3-10 min after an establishment of perforated patch-clamp recording. Then, test stimulation was paired with postynaptic depolarization at 1 Hz for 1 min. In 11 of the 15 cells in which the membrane potential was depolarized to -20 mV, LTP of EPSCs was induced following pairing. LTP of EPSCs was observed in 5 of the 14 cells depolarized to -40 mV. No LTP was observed when the postynaptic cells were clamped at -60, -70 or -90 mV. Thus, the induction of LTP may depend on postynaptic depolarization more than about -40 mV. Significant LTD was not induced in any of the cells tested with pairing stimulation of neighboring 2/3 cells. A question of whether this is also the case in synapses from layer 4 neurons to layer 2/3 neurons is being addressed with similar pairing procedures.

551.14
Synaptic transmission between descending fibers and lumbar motoneurons of the frog: Facilitation and long-term depression of single-fiber EPSPs. A.E. Dietz*, Y. V. Kushto*, and I.E. Clamann, Dept. of Physiology, Univ. of Bern, CH-3012, Switzerland, Inst. of Evolutionary Physiology & Biochemistry, 195272 St. Petersburg, Russia 
Twenty-five data sets of monoevoked EPSPs evoked by intracellular stimulation of descending fibers of the ventral lateral column were analyzed. Reticulospinal fibers (n=13) were identified by stimulation of the reticular formation; the remaining fibers (n=12) were presumably propriospinal. In both subsets of data there was considerable variability in EPSP mean amplitude (from 44 to 410 microV) and shape of averaged EPSPs (half-width from 4.9 to 23 ms). Amplitude of the monoevoked EPSPs was higher; in 4 cases mean amplitudes were bigger than 1 mv. There was strong positive correlation between the time/amplitude correlation between the time-amplitude correlation (for EPSPs with amplitude < 1 mV), implying variability in location of synaptic boutons. When a test stimulus was applied to a single fiber 40 ms after a conditioned stimulus, it produced weak facilitation of reticulospinal EPSPs (ratio between mean amplitudes of test and conditioned EPSPs was 1.218±0.22). For the propriospinal fibers (mean ratio 1.05±0.26) facilitation was observed in 38% of the cases. The short-lasting pathway was facilitated in 100% of the cases. In the remaining synapses, which all had high amplitude EPSPs, there was weak depression of the test EPSP: Simultaneous of a descending fiber at 5 Hz produced strong depression of the EPSP in 1.2 min. Recovery time after 5-10 min of stimulation was from 15 to 30 min (n=6) and in one case recovery was not complete 45 min after termination of 5 Hz stimulation. Ratio between amplitudes of test and conditioned EPSPs was not changed but half-width was increased within 5 min after the stimulation. We suggest that preynaptic mechanisms mediate the depression, and the effect is stronger at more proximal synapses. Supported by SNF.

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551.15

This study was undertaken to examine both long-term potentization (LTP) and kindling-induced potentiation (KIP) in the same structures in freely moving guinea pigs. Guinea pigs were chosen because they exhibit a number of differences in electrical kindling as compared to rats. Male ad libitum, mixed strain guinea pigs were chronically implanted with bipolar stimulating and recording electrodes. Animals had stimulating electrodes implanted in either the olfactory bulb (OB) or lateral olfactory tract (LOT) and recording electrodes in the piriform cortex. Following a two week recovery period, a series of baseline input/output (I/O) response curves were generated. These were based on the application of rectangular 200 μsec pulses at 11 intensities thru the stimulating electrodes. The potentials evoked in the piriform cortex were analyzed for maximum peak and minimum valley amplitudes. LTP experimental animals received high frequency stimulation (HFS) trains once a day for a total of four days. Kindling experimental animals received a total of 10 stimulations, one per day, consisting of 1 msec biphasic square wave pulses at 60 Hz, for 2 seconds. In these animals the resulting afterdischarges (AD's) were marked and the convulsive behaviour scored. Post-kindling I/O's were recorded for several weeks. We report potentiation of the platform evoked responses to HFS and kindling of the LOT but not to HFS and kindling of the OB. Supported by NSERC and AHFMR.

551.16
A DEVELOPMENTAL EXAMINATION OF LTP IN FREELY-MOVING RATS. J.D. Bronzi, Jr., Austin-LaFrance and P.J. Morgans. Dep't of Engineering and Computer Science, Trinity College, Hartford, CT 06106.

This study examined the establishment, maintenance, and decay of LTP across the perforant path/dentate granule cell synapse in freely-moving rats at 15, 30, and 90 days of age. Measures of population spike amplitude (PSA) and population EPSP slope were used to assess the magnitude and duration of LTP obtained from each age group. At 15 days of age, significant potentiation of both EPSP slope and PSA measures was obtained 15 min. after tetanization. This enhancement was maintained without significant change over the first 24 hrs. post-tetanization, after which these measures rose steadily during the 5 day recording period. To determine what percentage of this rise resulted from tetanization and what percentage resulted from normal development, input/output curves were recorded daily from non-tetanized pups as they matured from PND 16-29. Subtraction of developmental increases indicated that tetanization effects decayed to baseline between days 4 and 5 post-tetanization. At both 30 and 90 days of age, significant enhancement of both EPSP slope and PSA measures was obtained 15 min. after tetanization. These levels were significantly below those obtained from 15 day olds and were maintained without significant change for only 3-5 hrs., decaying to baseline within 24 hrs. Results indicate that LTP of both the EPSP slope and PSA can be reliably established in 15-day-old rats, and that both the magnitude and duration of this LTP is significantly greater than that obtained from juveniles or adults. The immature functional status of GABAergic interneuronal synaptic contacts with the granule cell population is hypothesized as a possible mechanism underlying both the greater magnitude and longer duration of LTP obtained from 15 day old animals. Supported by NSF Grant # BCS-9208128.

551.17
MOSSY FIBER LONG-TERM POTENTIATION IN CONSCIOUS, FREELY MOVING ANIMALS: TIME COURSE AND INDUCTION PARAMETERS. E.J. Barra-Rodriquez, E.B. Derrick and J.L. Martinez, Jr. Department of Psychology, University of California, Berkeley, CA 94720.

Previously we reported that in the anesthetized animal, mossy fiber long-term potentiation (MF-LTP) is frequency dependent (J. Neurosci., in press), because a minimum of 30 pulses at 100 Hz are needed to induce MF-LTP. In the present study, we investigated the stimulation parameters necessary for the induction of MF-LTP in conscious, freely moving animals with indwelling electrodes. We found that unlike the anesthetized preparation, 30 pulses at 100 Hz did not induce MF-LTP but that seizures were induced. By contrast, when the animals were placed in a novel environment, the administration of two 100 Hz trains induced MF-LTP, without the occurrence of seizures. This potentiation decayed within 24 hrs. We propose that the physiological mechanisms associated with hippocampal theta activity which are present in novel environment, prevent the occurrence of seizures and facilitate the induction of MF-LTP.

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551.18

Evidence that neural activity may cause the formation of synapses in the adult brain has come from studies of limbic seizures and kindling in the rat hippocampus (TINS 13: 312-318, 1990; J. of Neurosci. 9: 2795-2803, 1991). It has been shown that long term potentiation (LTP) of mossy fiber responses in area CA3 of the rat hippocampus constitutes an opioid receptor-dependent form of hippocampal synaptic plasticity (Brain Res. Bull., 27: 219-223, 1991). In the present study, Timm sulfide silver technique was used to examine whether LTP of mossy fiber-CA3 synapses affects structural reorganization and synaptic formation in the adult rat hippocampus in vivo. Our results show that seven days after LTP induction, a prominent band of Timm's staining is present bilaterally in the infrapyramidal band but in more prominent in the contralateral CA3 (stratum oriens). In addition, we found that naloxone (an opioid receptor antagonist) reverses this effect. Stimulation induced increases in Timm's staining, not associated with LTP, was also evident. The increase in Timm's staining which we interpret to be synaptic reorganization, becomes apparent as soon as 1 hour after the LTP induction and is quite pronounced after 7 days. These results indicate that synapses are formed in response to neural activity associated with LTP thus, experience-dependent information storage could involve local, activity-dependent synaptogenesis.

Supported by DA04195 to JLM, Rennie Fund of the University of California, F32-MH10641 to EJBR and CONACyT of Mexico to MLE.
552.1 IDENTIFICATION OF A NOVEL G-PROTEIN COUPLED RECEPTOR IN THE VENTRAL TEGUMENTUM BY PCR. M.L. Charlton* and R.S. Duman. Laboratory of Molecular Psychiatry, Deps. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06520.

We previously described the PCR-based identification of cDNA fragments encoding novel G protein-coupled receptors in the rat brain (Soc. Neuro. 19 Abstract #334). PCR of cDNA derived from the ventral tegmentum with degenerate primers to the third and sixth transmembrane domains (Liberti et al., Science 244:599, 1989) resulted in the amplification of several previously identified (e.g. beta-adrenergic, dopamine D2, neuropeptide K) as well as several novel PCR clones. One of these clones, VT-15, was chosen for further study. Sequence analysis revealed that this cDNA fragment is most homologous at the nucleotide and amino acid levels to the bombesin receptor (approximately 40%). Northern blot analysis of rat spleen RNA detected a single major RNA species of approximately 2.0 kb in length. In situ hybridization of rat spleen RNA detected a single major RNA species of approximately 2.0 kb in length. The relative abundance of this clone was determined by ribonuclease protection assay: levels of VT-15 mRNA were highest in the spleen, lung, heart, and liver with lower levels in the thalamus, pons, frontal cortex and hippocampus. In addition, VT-15 mRNA was also detected in neuroblastoma (focus coeruleus-like) and hematopoietic (HL-60) cell lines. The cellular distribution pattern of this clone is being determined by in situ hybridization. We are currently in the process of isolating a full length clone encompassing VT-15 to a rat spleen cDNA library. Further study will include characterizing the ligand specificity, pharmacology, and regulatory properties of the receptor.

552.3 MOLECULAR CLONING OF A NOVEL CANDIDATE G PROTEIN COUPLED RECEPTOR FROM RAT BRAIN. Z. H. Song, W. S. Young, M. J. Brownstein and T. L. Bonner. Lab. of Cell Biology, NIH, NIH, Bethesda, MD 20892.

To clone novel G protein-coupled receptors, degenerate primers designed from the regions conserved between SKR6 (a cannabinoid receptor) and other G protein-coupled receptors (GPCR) were used. The resulting contigs were digested with restriction enzymes selected to initiate cloning from other receptors, were used to amplify the DNA prepared from a rat cerebral cortex cDNA library. cRNA, one of the clones generated by PCR, had amino acid sequences that are typical of G protein-coupled receptors. Using a radiolabeled PCR fragment of cRNA as a probe, a full-length cDNA was isolated from the rat cerebral cortex library. This 1.8 kb full-length cDNA of cRNA was shown to encode a 1086 base pair open reading frame, encoding 363 amino acid residues. Sequence analysis demonstrated that cRNA possesses a number of structural characteristics of rhodopsin-like G protein-coupled membrane receptors, including seven putative hydrophobic transmembrane domains and six connecting loops. Comparing the sequence of cRNA with other G protein-coupled receptors revealed that cRNA is similar to GPCR1 and GPCR81 (61% amino acid identity in transmembrane region one through carboxy-terminal), two other published orphan G protein-coupled receptors. Therefore, cRNA, together with GPCR1 and GPCR81, may belong to a novel G protein-coupled receptor subfamily with identical or closely related endogenous ligand. Northern and in situ hybridization experiments demonstrated that cRNA mRNA is present in the rat brain, with particularly high levels of expression in striatum and retinopetal cerebral cortex. The precise physiological role of cRNA awaits elucidation of its ligand and signal transduction pathways.

552.5 CHARACTERIZATION OF THE RAT CEREBELLAR ENDOCYTHERING ETG RECEPTOR USING THE NOVEL ANTAGONIST [125I]BQ23020. M.F. Jarvis*, A.A. Assaf and E.G. Martin, Rhone-Poulenc Rorer Central Research, Collegeville, PA 19426.

The complex binding of radiolabeled endothelins isopeptides (ET-1, ET-2, ET-3) in rat brain of multiple affinity subtypes of the ETG receptor. Vasodilation has been associated with an interaction with a "super high affinity" ET receptor subtype whereas vasoconstriction is mediated via a subsequent "high affinity" receptor subtype. Recently, a low peptide fragment of ETA, N-acetyl-[Ala11,12]-ET-1(6-21) (BQ23020) has been identified as a potent and ETG-selective antagonist. The present studies were conducted in order to characterize the [125I]BQ23020 binding to the ETG receptor in rat cerebellum. [125I]BQ23020 (0.1 nm) bound with high specificity (90% of total) and selectively for the ETG receptor. ETA, ET-2, and ET-3 inhibited 0.1 nm [125I]BQ23020 binding with equivalent affinity (Ki values = 55 - 119 pm). The sarafotoxin S6a, S6b also potently inhibited [125I]BQ23020 binding (Ki values = 55 - 2000 pm). The ETG selectivity antagonist, BQ123 [10 μM] did not significantly inhibit [125I]BQ23020 binding. The selective ETG neuronal cDNA library studies indicated [125I]BQ23020 bound a single class of recognition sites with very high affinity (Kd = 31 pm) and limited capacity (Bmax = 570 fmol/mg protein). High affinity 0.1 μM[125I]BQ23020 binding in the cerebellum includes a broad spectrum of guanine nucleotides. ET-1 was found to produce a biphasic inhibition curve in competing for 0.5 nm[125I]BQ23020. This ET-1 inhibition curve was made monophasic by the addition of 1 μm the selective ETG receptor agonists. The present study indicates that ETG nervous system affinity and nucleotide sensitivity of [125I]BQ23020 binding suggests that the different functional consequences of ETG agonist activation may be mediated by different affinity states of the receptor.
PHARMACOLOGICAL CHARACTERIZATION OF RAT AND HUMAN CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS EXPRESSED IN STABLE CELL LINES

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Over the past decade, considerable evidence has accumulated from both laboratory and clinical studies implicating corticotropin-releasing factor (CRF) as a physiological mediator of stress responses or stress-induced disorders. Recently, using an expression cloning approach, two CRF receptors (CRF1 and CRF2) were isolated from a human ACTH-secreting pituitary adenoma. Full length human and CRF receptor cDNAs were subcloned in the mammalian expression plasmid pCDM-7 containing the human cytomegalovirus (CMV) promoter or a modified version of pCDM-7 in which the CMV promoter was replaced with the RSV promoter. These constructs were co-transfected with pSV2neo into two different cell backgrounds, COS-7 and Ltk-, which do not normally express CRF receptors, using the calcium phosphate precipitation method resulting in the generation of stable cell lines. CRF receptors expressed in these cell lines demonstrated saturable, stable, high-affinity binding to CRF with the pharmacological and functional characteristics comparable to those found in a variety of animal or human tissues. (125)I-CRF bound to a single population of binding sites in Ltk- cells transfected with human and rat CRF receptors with apparent affinities (Kd) of 130 and 168 pM and receptor densities of 97 and 588 fmol/mg protein respectively. The pharmacological rank order of potencies of CRF and related peptides was identical to the established profile for the CRF receptor in the rat frontal cortex. In addition, CRF receptors transfected into these cell lines were coupled to a guanine nucleotide binding protein and when incubated with Ca(2+) could stimulate the production of cAMP from these cells with an EC50 of approximately 1 nM. Furthermore, CRF-stimulated cAMP production could be inhibited by specific and selective antagonists to the CRF receptor. The stable expression of CRF receptors in clonal cell lines represents an unique opportunity for the discovery of non-peptide agonists or antagonists to the human CRF receptor for the possible treatment of CRF-mediated disorders.

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Corticotropic releasing factor (CRF), a 41-amino acid peptide, plays a significant role in integrating the stress-related and inflammatory responses to immune challenges such as viruses, bacteria, or tumor cells through its coordinated actions in the nervous, endocrine and immune systems. In addition to its effects on the hypothalamic-pituitary-adrenal (HPA) axis, CRF has recently been reported to regulate inflammatory responses. In this study, we have demonstrated that CRF has been described to enhance pro-inflammatory cytokine production. In vivo, anti-CRF antibody treatment was shown to lessen the recruitment of cells to an inflammatory site. In an effort to better understand the mechanism(s) of action whereby CRF may be involved in inflammation, CRF-receptor binding studies were carried out on immune cell populations. Specific, saturable high affinity binding of CRF to human peripheral blood mononuclear cells and monocyte lines using RT-PCR. Given that peripheral mononuclear cells express CRF receptors, further studies are underway to determine which immune cell populations express CRF receptors, and whether these receptors are modulated during an immune response, and if so, is receptor modulation due to pro-inflammatory cytokine production? These studies are likely to further our understanding of the role of CRF in inflammation.

EVIDENCE OF FUNCTIONAL GROWTH HORMONE-RELEASING FACTOR RECEPTORS IN RAT HYPOTHALAMUS. J. Boulanger* and P. Gaudreau
Neuroendocrinology Laboratory, Notre-Dame Hospital Research Center and University of Montreal, Montreal, Canada, H2L 4M1.

Recent reports suggest the existence of GRF receptors mediating endocrine and behavioral actions of this peptide, in the hypothalamus. Our goals were to characterize GRF binding sites in hypothalamic homogenates and to determine if this binding leads, as in the anterior pituitary, to the stimulation of adenylate cyclase (AC) activity. In our assay conditions (Tris buffer pH 7.4 containing MgCl2, EDTA, sucrose, dipotrite and BSA, 200 µg protein, 1 h, 4°C), 125I-GRF(1-44)NH2 was stable and specific binding represented 40-50% of the total binding. This binding was temperature and time-dependent, reversible and saturable. The affinity was hGRF(1-29)NH2 > NeAc-[his6-D-Arg9] > [Ala1] = [Ala1] = VIP and PACAP were inactive at all concentrations. In our AC assays containing [3H]cAMP, GRF(1-29)NH2 elicited a concentration-dependent production of cAMP. Although the efficacy of GRF was much lower in the hypothalamus than in the pituitary, its potency was not different in both tissues (EC50, NeAc-[his6-D-Arg9]>GRF(1-29)NH2 did not stimulate AC activity, but substantially inhibited the response to GRF(1-29)NH2). Although these results suggest the existence of functional hypothalamic GRF receptors, they still have to be localized to establish their involvement in the regulation of GRF secretion and control of appetite.

CELLULAR UPTAKE OF INTRACEREBROVENTRICULARLY INJECTED ANTISENSE OLGODEOXYNUCLEOTIDES INTERNALLY LABELLED WITH BODION OR DIGOXIGENIN. Y. Yue, H. Ericson, D.J. Reis and C. Wahlstedt, Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell University Medical College, New York, NY 10021.

Recently, we have demonstrated that intracerebroventricular (i.c.v.) administration of antisense oligodeoxynucleotides (ODNs) specific for the rat neuropeptide Y (NPY)-Y1 receptor and N-methyl-D-aspartate (NMDA)-R1 receptor subunit resulted in the inhibition of the respective binding in rats (Wahlstedt et al., Science, 259:528, 1993; Wahlstedt et al., Nature, 365:260, 1993). In addition, treatment with the NPY-Y1 antisense ODNs was shown to produce anxiolytic-like effects in the NMDA-R1 antisense ODNs reduced focal icischemic infarctions in rats. In contrast, the intracerebroventricular (i.c.v.) administration of antisense oligodeoxynucleotides (ODNs) specific for the rat neuropeptide Y (NPY)-Y1 receptor and N-methyl-D-aspartate (NMDA)-R1 receptor subunit resulted in the inhibition of the respective binding in rats (Wahlstedt et al., Science, 259:528, 1993; Wahlstedt et al., Nature, 365:260, 1993). In addition, treatment with the NPY-Y1 antisense ODNs was shown to produce anxiolytic-like effects in the NMDA-R1 antisense ODNs reduced focal icischemic infarctions in rats. In contrast, the intracerebroventricular (i.c.v.) administration of antisense oligodeoxynucleotides (ODNs) specific for the rat neuropeptide Y (NPY)-Y1 receptor and N-methyl-D-aspartate (NMDA)-R1 receptor subunit resulted in the inhibition of the respective binding in rats (Wahlstedt et al., Science, 259:528, 1993; Wahlstedt et al., Nature, 365:260, 1993). In addition, treatment with the NPY-Y1 antisense ODNs was shown to produce anxiolytic-like effects in the NMDA-R1 antisense ODNs reduced focal icischemic infarctions in rats. The stability of the injected antisense ODNs was examined by performing recovery experiments and by examining the potential for degradation of the oligonucleotide to antigenic response in rat brain extracts.
552.13
EXPRESSION OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE RECEPTOR SUBTYPES IN SUPERIOR CERVELAL GANGLION NEURONS. K. M. Brass* and V. May, Dept. of Anatomy & Neurobiology, Univ. of Vermont Coll. of Medicine, Burlington, VT 05405.

Pituitary adenylate cyclase activating polypeptides (PACAP) belong to the vasointestinal polypeptide (VIP)/secretin/glucacon family of bioactive peptides. The PACAP type I receptor is specific for PACAP, while the type II receptor is specific for PACAP and VIP. Splice variants of the type I PACAP receptor differentially stimulate adenylyl cyclase and phospholipase C. The PACAP peptides exerted potent regulation of superior cervical ganglion (SCG) neurons (y(NPY) and cAMP) in a dose-dependent manner. PACAP-induced sustained secretion of NPY and CA from cultured rat SCG neurons; neurosecretory responses to PACAP were approximately 100-fold more potent than VIP. To identify the population of responsive SCG neuron binding was determined using biotinylated PACAP38. Over 60% of the SCG neurons were labeled and may be responsive to PACAP regulation. To distinguish the expression of type I PACAP receptor isoforms, the presence or absence of either one or two 84 bp cassettes was examined using reverse transcription and polymerase chain reaction (RT-PCR) with mRNAm from fetal, neonatal, adult and cultured SCG cells. The predominant molecular form of PACAP receptor mRNA identified by RT-PCR contained one 84 bp cassette; further hybridization studies are necessary to identify the specific insert. No PCR products were observed using primers specific for the VIP (type II) receptor. These studies demonstrate that SCG neurons express specific type I PACAP receptor mRNA, bind PACAP and respond to PACAP stimulation. Supported by HD-27468.

552.15

Adrenomedullin (AM), a 52 amino acid peptide, has been recently discovered from human pheochromocytoma which has a hypertensive effect via an elevation in blood pressure. AM is located in various tissues other than pheochromocytoma such as brain, lung, kidney and structurally homologous to the calcitonin gene-related peptide (CGRP) and amylin. The peptides known to exert its effects via increasing cAMP is knowndiffusely, however, for the receptors for AM. We have reported that the protein encoded by the cystic fibrosis transmembrane conductance regulator gene (CFTR), the Cl- channel activated by A kinase, can be used as a sensitive receptor for cAMP changes in Xenopus oocytes (Receptor and Channels 1:235 (1993)). In the present study to see whether brain expresses the AM receptor, we injected cDNA for the CFTR along with poly(A)+ RNA from rat brain into oocytes. Three days after injection, rat AM resulted in a concentration-dependent increase in Cl- current via CFTR channels with the maximal response at 10^{-6} M. In the same oocyte, both CGRP and amylin 10^{-7} M also caused CFTR currents. The potencies for the CFTR currents of the three peptides were CGRP > AM >> amylin. The present results suggest this is a novel method for functional expression cloning of the receptors; although it is not known whether all these receptors have their own receptors or share several types of common receptors.

552.17
TRANSIENT CEREBRAL ISCHEMIA INDUCES OVEREXPRESSION OF OXYTOCIN RECEPTORS IN THE GERIL HIPPOCAMPUS. M. Dubois-Dauphin*, C. Brureau, and P. Valtier, Dept. of Physiology, University Medical Center, 1211 Geneva, Dept. of Psychiatry, Div. of Morphology, 1205 Geneva, Switzerland.

Using an iodinated oxytocin (OT) antagonist and light microscopy autoradiography, oxytocin receptors were detected in the brain of adult male gerbil following transient cerebral ischemia (6 min). Either immediately or 30, 100, 240 and 300 min after reperfusion and 24 h after ischemia, oxytocin receptors were detected in the CA1 and CA3 pyramidal regions in many brain areas. However, on the second or third day after ischemia, oxytocin receptor binding was increased in all brain areas. The increase in CA1 and CA3 pyramidal regions in many brain areas was followed by a decrease in the number of oxytocin receptors in the CA1 and CA3 regions after 72 h. These results suggest that transient cerebral ischemia induces the overexpression of oxytocin receptors in the hippocampus. The mechanism of oxytocin receptor expression after ischemia is under investigation.

552.18

Brain OTRs have been implicated in a variety of behaviors, e.g. sexual and parental. Previous characterization of peripheral OTRs has suggested diverse binding properties of OTRs in different tissues. Little is known, however, about the functional properties of rat brain OTRs. In the present studies, the pharmacological profile of rat brain OTRs was assessed. Brains from 10-day-old rats were homogenized and the membrane with the radioligand 125I-ori-motrine vasotocin ([125]OT) saturation studies with Scatchard analysis determined the density and affinity of brain [125]OT-OTVA binding sites (Bmax = 24 fmol/mg protein; Kd = 50 pm). The specificity of binding was confirmed by competition with unlabelled OT (Kd = 2.01 ± 0.08 nm, n = 5). The rank order potency of brain OTR agonist binding was OT > APV > thrpGly6-ot (n = 3). OT binding was best fit with a two-site model, with approximately 50% high affinity sites (Kd = 2.12 ± 0.42 pm, Bmax = 11.1 ± 2.1 fmol/mg protein) and approximately 50% low affinity sites (Kd = 10.80.0 mg protein). Preliminary studies using nonhydrolyzable analogs of GTP, GTPγS, resulted in monophasic OT binding with the low affinity component, which indicates that brain OTRs, like their peripheral counterparts, are coupled with G-proteins. *Supported by NS23986 and MIA43787.
555.3

CORTICOTROPIN-RELEASING FACTOR (CRF) INCREASES HIGH-VOLTAGE ACTIVATED (HVA) CALCIUM CURRENTS IN ACutely DISASSOCIATED NEURONS OF THE CENTRAL AMYGDALA (CeA). B. Yu* and P. Shamrock-Gallagher. Dept. of Pharmacology & Toxicology, Univ. of Texas Medical Branch, Galveston, TX 77555.

Slices of the CeA mediate stress responses in the brain. We tested CRF on calcium currents in CeA neurons. When held at 80 mV, HVA currents peaked at +10 mV and averaged 289±17 nA/pF (n=18). Intracellular loading of 26Ca with Fura-2,3-AM (IC 50≈5.6% of 2Ca current) did not reduce HVA currents. Moreover, over 80% of CeA neurons possessed L-type HVA currents. Δ,CTX SV5 (1-2 μM) inhibited 24%±6% of the peak I_{Ca,L}, indicating the presence of voltage-gated L-currents. Δ,CTX MVIC (200 μM) inhibited 30%±4% of the peak I_{Ca,L}, suggesting the presence of Q-type currents. No P-type current was recorded since Δ,Agateoxin (100-200 nM) did not reduce I_{Ca,L}. These data suggest that the HVA calcium currents of CeA neurons are comprised of N, L, and Q but not P-type of calcium currents. CRF (1-400 nM) enhanced HVA calcium currents in approximately 50% CeA neurons recorded. However, in the remaining neurons, CRF had no effect. CRF enhanced both peak and steady state HVA calcium currents. CRF (50 nM) increased the peak HVA-2.5 μA/cm² (n=6). No shift was observed in the peak of the current-voltage relationship. The current-increase in HVA calcium currents is consistent with CRF antagonist ability to inhibit CeA neuron receptor antagonists, which antagonize the GABAergic transmission. These data suggest that CRF enhances calcium currents through a receptor mechanism and could thereby facilitate neuronal communication through CeA neurons during the stress response. (Supported by NS 29265.)

555.4


We previously showed that IC CRF (21 and 63 pmol) decreased GVED by 17.4 and 24.0% respectively in rats. Urethane induced an increase of hypothalamic CRF transcripts and secretion and low basal activity of GVED. Thus, to assess the influence of the new CRF antagonist, [DPhe^2,Leu^5,6-Cr(CrEt)Leu^7,10]-CRF_{29-41} given IC on GVED in urethane-anesthetized rats. Male Sprague-Dawley rats (280-320 g) were injected with urethane (1.5 g/kg i.p.) and implanted with a catheter into the cisterna magna for IC[15] a i.a. injection. A strain of the ventral gastric branch of the vagus was cut distally to record multi-unit efferent discharge. Nerve signals were sent to a window discriminator and counted on computer. Maximum 1 min peaks from basal GVED were calculated and expressed as Mean±SEM peaks from baseline. 10 IC injections of CRF (15 μl) did not change CRF antagonist, 5/[Cr(CrEt)Leu^7,10]-CRF_{29-41} (25 μg/s) over 15-30 sec were performed at 10-15 min intervals and thereafter CRF was injected IC. Recall 1 IC vehicle (n=11) had no significant effect on basal GVED (101±6 μl, 4.8±0.05 μl/min). Maximum 1 min peak from CRF antagonist was obtained at 24.5±3.8% 3 min after injection. The excitatory response peaked within 1±2 min (3.8±0.2 min). In contrast, a maximum 1 min peak from vehicle was obtained at 30.0±3.3% 1 min after injection. 24.4±1.0 μl/min after injection. These results suggest that the new CRF antagonist is effective in GVED, while the injection of CRF (63 pmol/rat) at 15±1 min after the CRF antagonist produced a significant inhibition of GVED by 82.2±5.4% (n=6). Conclusions: These results suggest that the new CRF antagonist may be of potential interest in the treatment of functional and organic dysfunctions of the efferent gastric motor nerve pathways. (Supported by NIH grants NS38433 & DK51110.)

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THURSDAY AM PEPTIDE RECEPTOR STRUCTURE AND FUNCTION IV

555.19

PEPTIDE AND CORTICOTROPIC RELEASING HORMONE mRNA EXPRESSION IN COLD STRESSED RATS. S. Makio1, K. Fukuhara2, M. A. Smith3, and P. W. Gold1. Clinical Neuroendocrinology Branch,1 Biological Psychiatry Branch,2 NHIM; Clinical Neuroscience Branch2; NINS; National Institutes of Health, Bethesda, MD 20892.

Exposure to cold is a potent stimulus of the hypothalmo-pituitary-thyroid axis. However, the role of CRF and related peptides in cold stress has not been established. To address these issues, we exposed rats to cold (3°C) continuously for 0.5, 1, 3, 6, 24 hours and 5 days, and examined CRH mRNA expression in the paraventricular nucleus (PVN), central nucleus of the amygdala (CeA) and bed nucleus of the stria terminals (BNST) by using in situ hybridization histochemistry. Plasma levels of corticosterone increased transiently at 0.5 h, then returned to normal by 1 h. CRH mRNA in the PVN and POMC mRNA in the anterior pituitary did not change up to 5 days. This indicates that cold stress may transiently induce CRH or ACTH secretion, but may not be enough to stimulate CRH or ACTH synthesis. By contrast, CRH mRNA in the CEA and dorso-medial division of the BNST decreased at 6 and 24 hours exposure to cold (maximally by 25% and 45% at 24 h) compared to control in the CEA and BNST, respectively. These data suggest that (1) cold is a mild stressor, if any, of the HPA axis, (2) but that cold stress can modulate extra-hypothalamic CRH.
553.5 SOMATOSTATIN EFFECT ON GUINEA-PIG DORSAL THALAMIC NEURONS IN VITRO. B. Rat* and R. Limas, Dept. of Physiology & Biophysics, New York University Medical Center, 550 First Avenue, New York, NY 10016.

Somatic and voltage-evoked electroresponsiveness were examined using sharp microelectrode intracellular recordings in dorsal thalamic neurons in guinea-pig slices. A few minutes following bath application of 2 μM somatostatin superfusion, a characteristic increase in low-threshold calcium spike activity was noticed in specific thalamic nuclei. The results could be observed, at the spontaneous-electrical-activity level, as a marked tendency towards intrinsic oscillation, often occurring from a membrane potential similar to the initial non-oscillating level, where low-threshold activity was only observed after membrane hyperpolarization in the order of 5 to 10 mV. In other cases the increase of oscillatory activity would occur on depolarizations or hyperpolarizations of a few millivolts from the initial resting-potential level. The results suggest that such changes in the resting level of the cell, which are either too small, or in the wrong direction, to affect directly the level of voltage-dependent inactivation of the Type-I calcium channels responsible for such low-threshold activity were not the decisive factor affecting the excitability changes.

Similar results were obtained by using neuronal excitability directly, via transmembrane current pulses to evoke different firing potentials. In somastostatin, the voltage dependence for low-threshold calcium-spiking induction was found to decrease by close to 30% with reference to the normal voltage level at which low-threshold spikes are generated. The results suggest that the level at which low-threshold calcium spikes is triggered in thalamic neurons may be regulated by means other than transmembrane voltage. Somatostatin did not affect all thalamic neurons, moreover cells that responded were localized in a group probably related to somatostatin innervations.

The results indicate that neuropeptides may exercise regulation of intrinsic membrane properties onto well-defined sets of thalamic nuclei without affecting others, and so, may modulate global brain activity by acting on the thalamocortical system in a manner different from that generated by conventional synaptic input.

NIH NS31374.

553.7 EFFECTS OF CYSTEAMINE ON REGIONAL CHOLINE ACETYLTRANSFERASE ACTIVITY IN RATS. K. Koku, H. Kanda, Y. Komurasaki, K. Saka*, and K. Makeda, Dept. of Psychiatry, Kobe Univ. Sch. of Med., Kobe, Japan.

It is well known that somatostatin (SS), one of brain neuropeptides, is decreased in cerebrospinal fluid or postmortem brain with Alzheimer's disease. Dysfunction of cholinergic neurons in Alzheimer's disease is also reported. The relation between SS and cholinergic system is not obvious yet in Alzheimer's disease. In this study, we investigated the effects of cysteamine, which depleted brain SS contents, on regional choline acetyltransferase (CAT) activity in rats.

Male Wistar rats (300-350 g) were used. Cysteamine was dissolved in saline and adjusted to pH 7.4. 150-300 mg/kg cysteamine and saline for controls were injected intraperitoneally (i.p). Four hours later, rats were decapitated and the brains were dissected into the olfactory bulb (OB), anterior cortex (Ac), temporal cortex (Tc), parietal cortex (Pc), hippocampus (Hi) and striatum (St) with Glower's method. SS and neuropeptide Y (NPY) contents of each region were measured by radioimunossay and CAT activity by Fouassier's method. Statistical analysis was performed by Student's t-test.

As a result, SS contents in the hippocampus were only decreased significantly (20% of mean of saline was 33.8 ± 1.6; 29.7 ± 1.2, 18.4 ± 2.4 % at 90, 150, 300 mg/kg, p < 0.05). However, NPY contents and CAT activity did not have any changes in any region.

Our results suggest that cysteamine may not have effects on CAT activity directly in the brain regions and the decrease of hippocampal SS contents may not, too.

553.9 DOPAMINE-INDUCED INHIBITION IS ATTENUATED BY IONTOPHORESICALLY APPLIED NEUROTENSIN IN THE NUCLEUS ACCUMBENS OF RATS - IV VIVO STUDY. Z. Z. Sternow*, J. C. Landers, Z. I. Tang, and C. B. Nemeth*. Laboratory of Neuropsychopharmacology, Dept. of Psychiatry and Beh. Sciences, Emory Univ. Sch. of Medicine, Atlanta, GA 30322.

Neurotensin (NT), an enogenous tridecapeptide, possesses a pharmacological profile that is similar to antipsychotic drugs. Previous electrochemical data suggest that both direct and intracerebroventricular (ICV) application of NT produces predominantly excitatory responses in dopamine (DA) neurones in the ventral tegmental area (VTA). The administration of NT consistently antagonized DA-induced inhibition in the VTA. In contrast, McCarthy et al. (Gen Pharmacol. 10 333-337,1979) found that intracerebroventricular application of NT (10 μg) had no effect on the firing rate of DA-sensitive neurones in the nucleus accumbens (NA).

The effects of iontophoresically applied NT on DA-induced inhibition in individual neurones in the NA were examined. Extracellular neuronal recordings were obtained from spontaneously active neurones in the NA in chloral hydrate anesthetized (400 mg/kg, i.p) male rats (250-350 g). DA sensitivity was confirmed via iontophoretic application of the racemate (25-35 μg) of DA (0.1M, pH 4.8). A total of 12 neurones were included in the data analysis [percent change = ΔTS + DR (VTA)]. Direct iontophoresis (25-40 μA) of NT (0.5 μM, pH 4.8) did not produce any significant alteration in firing rates (μA) [percent change = 0.46±0.17%. However, direct application of NT antagonized DA-induced inhibition in all neurones tested (μA).

These findings provide electrophysiological evidence that NT antagonizes the effects of DA in the NA. These findings support the hypothesis that NT receptor agonists may prove to be useful adjuvants in the treatment of psychosis, particularly related to excessive mesolimbic DA tone. SR 48692 was a gift from D. Galley, Sandoz Research, France. Supported by the Scottish Rite Schizophrenia Research Program, a Dalhousie University from the American Philosophical Society to ZNS and NIMH MH-39415 to CBN.

553.6 SOMATOSTATIN (SS) EFFECTS ON CYTOSOLIC CALCIUM CONCENTRATION ([Ca²⁺]) IN PC12 CELLS. G. Traina, S. Cannistraro and P. Bagnoli*, Dept. of Environmental Sciences, Tuscia University, 01100 Viterbo and Dept. of Physiology and Biochemistry, University of Pisa, 56123 Pisa, Italy.

Recent findings suggest that, among neuropeptides, SS plays an important role in the modulation of neuronal cell functional activity. The control of [Ca²⁺], has long been recognized as a fundamental mechanism of cell activation. In the present study, the effects of SS on [Ca²⁺], have been studied by conventional fluorescence in PC12 cells loaded with Fluo-2 in the presence of 2μM Ca²⁺. In all the experiments, the application of either Ca²⁺ ionophores or high K⁺ concentration has been used to increase the [Ca²⁺]. Our results can be summarized as follows: i) the application of the tetradecapeptide form of SS, SS-14 (10⁻⁷-10⁻⁹M), induces a dose-dependent decrease of [Ca²⁺]; ii) similar effects are induced by the application of SS analogs (SMS 201-995; D-Trp,D-Cys-SS; D-Trp-SS; MK 678; CGP 29396); iii) pretreatment with a SS antagonist (Cyclo-SS) prevents the effects of SS-14 application. These observations suggest that SS may modulate nerve cell functional activity by a specific inhibition of Ca²⁺ channels. In addition, experiments with selective blockers of different voltage-dependent Ca²⁺ channels (D 600, ß-conotoxin, GVIA, nitrendipine, nifedipine) suggest that this inhibition is mediated by Ca²⁺-channels of the L-type.

553.8 SELECTIVE EFFECTS OF NEUROTENSIN IN THE PFRONTAL CORTEX FOLLOWING DOPAMINERGIC STIMULATION IN THE NUCLEUS ACCUMBENS. D. Mora* A. Drumheller and F. B. Jolisseur Departments of Pharmacology and Physiology, Faculty of Medicine, Univ. of Sherbrooke, Sherbrooke, Quebec, Canada. J1H 5N4.

Neurotensin has been shown to markedly reduce the behavioral hyperactivity which appears 2 hr after intra-accumbens administration of the potent dopamine receptor agonist, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydro- 

neurotensin [ADTN] (Jolisseur et al., Neuropeptides 6:143, 1985). In order to better characterize this finding, the effects of bilateral intra-accumbens administration of ADTN (2.5ug) on concentrations of neopyrinine (NE) and its metabolites DOPAC and HVA, serotonin (5-HT) and its metabolite 5-HIAA were examined in terminal regions of dopaminergic fibers, including prefrontal cortex, amygdala, septum, globus pallidus, striatum and the nucleus accumbens itself. Following ADTN administration, chemical changes were assessed at 2 hr, when animals displayed a marked hyperactivity. Results indicate that in-vivo administration of ADTN results, 2 hr later in significant decreases in concentrations of NE, DA, DOPAC and HVA as well as of 5-HT and 5-HIAA in all areas. In a follow-up experiment, neurotensin (3.5ug) was injected intracerebrally 20 min prior to the neurochemical determinations performed at 2 hr after intra-accumbens administration of ADTN. Except for the prefrontal cortex, neurotensin accentuated the observed neurochemical changes in all regions. In the prefrontal cortex, neurotensin also further reduced concentrations of NE, 5-HT and 5-HIAA; however, the peptide systematically reversed the decreases in DA, DOPAC and HVA produced by ADTN in this region. These findings suggest that: a) dopaminergic stimulation in the accumbens results in widespread and enduring neurochemical changes; and b) neurotensin appears to have a selective neurochemical action in the prefrontal cortex in these circumstances.
553.11 NEUROTENSIN REVERSES LOCOMOTOR INHIBITION PRODUCED BY MICROINJECTION OF DOPAMINE INTO THE MEDIAL PREFRONTAL CORTEX: R. A. Radulovic and Y. E. Raina. School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO 80262.

It has been postulated that increased dopamine (DA) activity in the medial prefrontal cortex (mPFC) exerts an inhibitory influence over DA release in the nucleus accumbens leading to a reduction in locomotor activity. In addition, it has been demonstrated that neurotensin (NT) modulates DA-stimulated cell activity in the mPFC (Beauchamp, M. et al., Neurosci. Lett., 473:613-619, 1992). Thus, experiments were designed to test two hypotheses: 1) that an increased density of mPFC DA autoreceptors and 2) that NT modulates DA-influenced locomotor activity in the mPFC. Bilateral cannulae guides were implanted into the mPFC in pentobarbital/chloral hydrate anesthetized LHDU mice. Open field behavior was monitored in 5 minute blocks for a total of 30 minutes 24 hours post surgery in an automated open field apparatus (Omnitech). The distance traveled within the first 15 minutes after injection of drug was monitored for analysis. The injection naive to the apparatus, were injected bilaterally with either GBR-12909, a selective DA uptake blocker, neurotensin, or a combination of the drugs. Drugs were delivered at a volume of 100 nl per side over a 30 second period and were prepared in normal saline. GBR-12909, in doses ranging from 30 ng to 1 umol per side, produced a U-shaped dose response curve. A maximum inhibition of 49% of saline-injected control values was observed at a dose of 3 ng. The highest dose tested of GBR-12909 (1 umol) caused the animals to become significantly activated to 129% of control values. Higher doses of GBR-12909 could not be tested owing to the insolvibility of the drug at higher concentrations. Doses of NT from 0.01 umol to 1 umol were tested and found to have no dose-related effect on locomotor activity. NT, when co-injected with 3 pgms GBR-12909, dose-dependently reversed the inhibitory effect of GBR-12909. A dose of 3 pgms NT completely abolished the locomotor inhibition produced by GBR-12909. In conclusion, these results are consistent with the hypothesis that the DAergic system of the mPFC regulates locomotor activity. Furthermore, these results suggest that NT modulation of DA function in the mPFC may be implicated in the regulation of locomotor activity. (This work was supported, in part, by USPHS grants AA07097 and AA07330.)

553.13 CHOLECYSTOKININ- AND NEUROTENSIN-EVOKED CATIONIC CURRENTS IN SUBSSTANTIA NIGRA DOPAMINERGIC NEURONS ARE MEDIATED BY D1 AND D5 RECEPTORS: T. Wu and H. L. Wang. Dept. of Physiology, Chang Gung College of Medicine and Technology, Ketan-Sun, Tao-Yuan. 1 Dept. of Neurology, Chang Gung Memorial Hospital, Taipei, Taiwan 33345.

To understand the iononic and molecular mechanisms by which sulfated cholecysteinin octapeptide (CCK-8) and neurotensin (NT) modulate the electrical activity of dopamine neurons (SN DA neurons), whole cell patch-clamp recordings were used to study electrophysiological effects of CCK-8 and NT on acutely isolated SN DA neurons. Both CCK-8 and NT excite SN DA neurons via activation of nonselective cationic conductance. CCK-8 and NT-evoked inward currents were inhibited by the intracellular perfusion of GDP-beta-5 (1 mM). In DA neurons internally perfused with GTP-gamma-S (0.5 mM), the inward currents produced by CCK-8 and NT became irreversible. Pretreating DA neurons with 500 mM pertussis toxin (PTX) did not significantly affect the activity of both peptides to induce cationic currents. Intracellular application of heparin (2 mg/ml) (1.47 microM) of ligand to receptor antagonist, and buffering intracellular calcium with the Ca++-chelator BAPTA (10 mM) suppressed cationic currents evoked by CCK-8 and NT. Dialyzing DA neurons with protamine kinase (PKC) inhibitor, staurosporine and PKC9-13, failed to prevent CCK-8 and NT from generating cationic currents. It is concluded that PTX-insensitive G-proteins mediate neuropeptide-induced enhancement of cationic conduction of DA neurons. The coupling mechanism via G-proteins is likely to involve the generation of IP3 and subsequent IP3-evoked Ca++ release from the intracellular store results in activating the nonselective cationic conductance.


The C-terminal hexapeptide fragment of neurotensin (NT-8(13)) has been shown to bind to the neurotensin receptor and retain full agonist activity. Structure-activity studies on a variety of NT-8(13) mimetics resulted in the discovery of compounds which behaved as NT receptor agonist in vitro, and had a variety of behavioral effects consistent with NT agonist activity within the rodent system. NT-8(13) agonist (0.1nM-1uM) in the nucleus accumbens of the isolated perfused rat brain may have high affinity for the NT receptor found in new born mouse brain. This study was effective in inhibiting calcium mobilization (EC50 = 36mM). It's putative produg PD 149163S effectively inhibited spontaneous locomotor activity in the mouse (ED50 = 0.2 mg/kg IP) and acetic acid induced stretching (ED50 = 0.05 mg/kg IP). These effects were not reversed by naloxone or the NT antagonist SR 48692. PD 147113S was also shown to have potent systemic activity in the acetic acid induced stretching paradigm (ED50 = 0.08 mg/kg IP). These findings demonstrate systemic activity of NT agonists may have utility as antinociceptive, anesthetic or anorectic agents.

553.16 SUBSTANCE P (SP) AND THE SELECTIVE OPIOID AGONIST, DAMGO, ARE PHYSIOLOGICAL ANTAGONISTS WITHIN THE VENTRAL PALLIDUM (VP). L. Mignardi* and T. C. Nguyen. Department of Pharmacology, Loyola University Chicago, Stritch School of Medicine, Maywood, IL, 60153.

It has been demonstrated with various paradigms that SP and opioids are likely physiological antagonists (e.g., SP antagonists block behavioral symptoms of morphine withdrawal; enkephalins attenuate pain by blocking release of SP). Axons from the nucleus accumbens (NA) neurons that contain SP and enkephalin-like immunoactivity synapse on the VP neurons. Both SP and opioid receptors are found within the VP. Our previous work demonstrated that SP increases and opioids decrease firing rate of the VP neurons. To ascertain possible interactions between opioids and SP within the VP, the present study employed extracellular recording of unit activity and microinjection of DAMGO (10mM) and SP (1mM) in chloral hydrate anesthetized rats. Sixty-one VP neurons were tested with both compounds. Forty-eight percent of these were sensitive to both compounds, and 49% to SP. DAMGO microinjected into the nucleus accumbens and stratum of urethane-anaesthetized rats as measured by intracranial microdialysis (ICMD). In untreated animals, MNT 8-13 (2.5mg/kg s.c. ) produced variable changes in DA and DOPAC in both regions. However, in those animals pre-treated with pargyline (50mg/kg i.p.), DA levels increased 69% in the nucleus accumbens and 30% in the striatum, while DOPAC values remained relatively unchanged following MNT 8-13 injection. We conclude that under certain conditions, stable neurotensin analogues are able to preferentially modulate DA turnover in the mesolimbic system of rats following peripheral administration.
554.1 MODULATION OF INTRACELLULAR CALCIUM BY BRADYKININ IN N1E-115 CELLS. Jay S. Coggins* and Stuart H. Thompson. Department of Pharmacology, Division of Biological Sciences, Stanford University, Pacific Grove, CA 93950.

Murine N1E-115 neuroblastoma cells release calcium from IP3-sensitive intracellular stores in response to bradykinin (BK), but not the selective BK1 receptor agonist des-Arg9-BK. This suggests that the BK receptor type is expressed. Low concentrations (<500 pM) of BK elicited responses that are slow in onset (20-30 sec), long in duration (2-4 min) and exhibit low frequency calcium oscillations (20-50 sec periods). When N-acetyl-D-glucamine is substituted for extracellular Na in order to block NA/CA exchange, the calcium concentration in response to BK (500 pM) have shorter periods but are less profound. High concentrations of BK (1 μM) result in rapid rising calcium concentrations which smoothly decay to baseline within one minute in the continued presence of agonist. Short duration application of BK to 30 sec elicited similar calcium transients, and responses to subsequent applications exhibit marked desensitization. In the absence of extracellular calcium, the responses to bradykinin display the same characteristics. Trace averages from several cells indicate that there is no difference between responses in the presence or absence of extracellular calcium. Ca2+ influx, if any, is not associated with the release of calcium from intracellular stores in response to BK. Supported by NIH NS14519.

554.2 BRADYKININ-INDUCED OSCILLATIONS OF CYTOLIC CA2+ ACTIVITY AND MEMBRANE POTENTIAL IN RAT GLIOMA CELLS. G. Reeti and G. Reiter*. Institute of Physiological Chemistry, University of Tübingen, Hoppe-Seyler Str. 4, 72076 Tübingen, Germany.

In 2-load rat glioma cells (C6-4-2) continuously superfused with bradykinin (BK), rhythmic membrane depolarizations and calcium oscillations (50-200 Hz) were observed. The initial [Ca2+]i response to BK resulted from InsP3-induced Ca2+ release, whereas the subsequent oscillations were dependent on both InsP3-sensitive Ca2+ stores and Ca2+ influx. Simultaneous intracellular recording of the membrane potential showed that the oscillation of [Ca2+]i and of the membrane potential were synchronous. The oscillations were affected by the K+ equilibrium potential and by blocking of K+ (K5) channels. This indicates a potentiation of Ca2+ influx by membrane hyperpolarisation due to activation of K+ (K5) channels. Membrane quench experiments gave additional evidence for influx of Ca2+. We conclude that Ca2+ is periodically released from Ca2+ stores and subsequently opens K+ (K5) channels. The stores are refilled by the Ca2+-ATPase and by influx of Ca2+ across the cell membrane, augmented by membrane hyperpolarisation. The oscillations were also influenced by hypotonic and by hypertonic medium. Thus cell volume probably serves as a negative feedback regulator during the falling phase of [Ca2+]i. These experiments are discussed with respect to physiological functions of glial cells in volume regulation during ischemia or stroke.

554.3 EVIDENCE FOR A NEUROGENIC COMPONENT IN BRADYKININ BI RECEPTOR-MEDIATED PLASMA EXTRAVASATION DURING TUBERCULOSIS INFLAMMATION IN THE RAT. M.J.K. Alexander and L. Perkin*. Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6HN, U.K.

Although bradykinin (BK) receptors are not involved in the acute activation of nociceptive neurones, several studies have demonstrated that following persistent inflammation B1 receptors are induced and contribute to inflammatory pain. The present study investigates the role of these receptors in naive animals, but has been found to increase nociceptive responses following an inflammatory insult in rats. In addition to the sensitisation and activation of nociceptive afferents, kainic acid also mediates other symptoms of inflammation including oedema formation. The experiments presented here have investigated whether B1 receptors induced following inflammation also mediate plasma extravasation (PE). PE was examined in rats that had been injected with 5% dextran in a single hind paw 72 hours prior to testing, and was measured as the extravasation of 125I-BSA followed intraplantar injections of des-Arg9 BK. Des-Arg9 BK (100-300 pmol/paw) induced a significant increase in PE in both the ipsilateral and contralateral hind paws of turpentine-treated, but not naive rats. An increase in PE was observed 60 min., but not at 5 or 30 min, following des-Arg9 BK administration. This effect was reversed by the antagonist, des-Arg9 Leu8 BK. A neurogenic component of des-Arg9 BK-mediated PE was revealed by pretreating rats with capsaicin in order to deplete C-fibres. Capsaicin pretreatment reversed the B1-mediated PE, but had no effect on the baseline extravasation in controls. Inhibition of des-Arg9 BK-mediated PE by co-administration of a NK1 receptor antagonist, CP99994, suggests that B1 receptor-mediated PE involves the activation of C-fibres and the release of substance P. These results are consistent with a systemic induction of B1 receptors following local persistent inflammation. They also suggest that B1 receptor activation initiates a chain of events which ends with a neurogenic-mediated PE.


Substance P (SP) depolarizes some primary afferent neurones situated in trigeminal or dorsal root ganglia. This study represents an initial investigation of a SP-induced hyperpolarizing response to SP recorded in primary afferent neurones, those located in the nodose ganglion. In intact and in acutely dissociated neurones, 100 nM SP hyperpolarized the membrane potential by -70 ± 0.7 mV and decreased the membrane input resistance by 230 ± 110 MΩ. The hyperpolarization or outward current produced by SP was observed in 105 out of 132 cells (80%) and was dose-dependent (EC50 = 68 ± 11 nM). The SP effect was reversibly abolished by 10 nM CP96,345, an NK-1 antagonist, and was mimicked by 100 nM ADMSP, an NK-2 agonist. The response was not affected by 100 nM SR48969, an NK-2 antagonist. The SP effect was reversibly abolished by lowering extracellular Ca2+ (400 ± 76 mV/10-fold [K+]0). Our results show that SP activates an NK-1 receptor coupled to a Ca2+-dependent outward K+ current. We have observed SP-like immunoreactivity associated with ferret nodose neurones and therefore propose that SP receptors on these neurones may function as inhibitory autoreceptors.


Several studies suggest the existence of the tachykinin NK1 receptor subtype in the rat spinal cord. To clarify this issue and to study the possible involvement of NK1 receptors in synaptic transmission, the specific NK1 receptor antagonist SR48969 was used during intracellular recording from functionally-identified lumbar motoneurones (rest potential 74±2 mV, means±SEM) of the neonatal rat (P5-12) spinal cord continuously superfused in drugs were bath-applied. In all cells SR48969 (0.5 μM) had no effect on resting membrane potential or resistance but it did reduce the depolarization (50±10%, n=5), the increase in input resistance (80±7%, n=5) and the baseline excitatory activity induced by the specific NK1 receptor agonist [Asp9]NOE [200 nM]. SR48969 did not affect analogous changes produced by substance P-methylster (200 nM) and Met-enkephalin-β-endorphin (200 nM), specific for NK2 and NK3 receptors, respectively. For synaptic transmission studies two different types of dorsal root stimulation was used to recruit either AS fibers alone or C fibers as well as C fibers. AS fibers were employed to elicit slowly increasing depolarizations with slow recovery (windups). In the presence of SR48969 no change in response amplitude for all three types of stimuli except for modest reduction (10%) in the duration of the depolarization tail following windup was noted. Our study confirms the existence of SR48969 sensitive NK1 receptors in rat spinal cord and supports their role in neurotransmission from dorsal root afferents to motoneurones. Supported by CNR and INFM.

554.6 IS THE GALANIN-INDUCED INCREASE IN POTASSIUM CONDUCTANCE AND DECREASE IN BARBITURATE RESISTANCE OCCURRING IN MUDPUPPY PARASYMPATHETIC NEURONS MEDIATED BY TWO DIFFERENT RECEPTORS? L.A. Merriam*, J.M. Malvancy and R.L. Parsons. Dept. of Anatomy and Neurobiology, Univ. of Vermont, Burlington, VT 05405.

Galanin increases inwardly rectifying potassium conductance (gK) and decreases voltage-dependent barium currents (IBa) in mudpuppy parasympathetic neurones. In the present study it was determined that the concentration dependence of galanin and sensitivity to pertussis toxin and the chimeric ligand galantide to determine whether galanin's actions are mediated by the activation of a single receptor or different receptors. All experiments were done on dissociated neurones from the medulla of the mudpuppy Necturus maculosus using the perforated patch mode of the whole cell voltage clamp technique. We found that the EC50 for the activation of gK was -44 ± 4 nM and the maximum conductance was 75 ± 10% (n = 4) of the maximum conductance. The EC50 for the inhibition of IBa was -0.4 ± 0.1 nM. Pretreatment with PTX (-10 μM) for 24-36 hrs inhibited both actions of galanin. Galantide (10^-10M) antagonized the increase in potassium conductance by 10^-8M galanin without any measurable agonist action. In contrast, galantide (10^-10M) antagonized a concentration-dependent decrease in IBa. IBa could be further decreased by 10^-6M galanin in the presence of galantide. These results suggest that although activation of a G protein is involved in both actions of galanin, two different galanin receptors may mediate the increase in potassium conductance and decrease in IBa in mudpuppy neurones. Supported by NS23978.
BARRECTOR FLEX MODULATION BY THE GALANIN FRAGMENT (1-15) [D. A. Jennewijen*+], P. L. Heidtmann, J. L. A. Agster, N. Yamaoka, S. Gonzalez-Spín and K. Fujita. Dept. of Physiology, Faculty of Medicine, University of Salamanca, Spain. Lab. of Bio-Organoic Chemistry, School of Pharmaceutical Sci., University of Shizuoka, Japan and Dept. of Neuroscience, Karolinska Institutet, Stockholm, Sweden.

Recent studies have reported that the galanin (1-15) [GAL (1-15)] administered centrally elicits dose-related vasopressor responses and counteracts the vasodilator activity induced by galanin (1-29) [GAL (1-29)]. For a better understanding of the role of the galanin peptide family on central cardiovascular control we have studied the modulation of the baroreflex responses (BBR) by both galanin molecules. Groups of unanesthetized rats occupational in the lateral ventricle (i.v.:) of 0.1 nmol of GAL (1-15), 0.3 nmol of GAL (1-29), 3 nmol of GAL (1-29) and 20 pmol of the cholinergic agonist, methylcholine (MCh) were tested by an automatic injection pump (30 μL/min). BBR was elicited before and after i.v. injections by intravenous bolus injections of few different doses of L-phenylephrine (LPE) (33 to 625 μg/kg i.v.) and normetanephrine (NME) (10 μM) at heart rate (HR) changes were recorded from a catheter in the femoral artery. BRR sensitivity was evaluated from the slope of the regression lines related to the maternal reflex bradycardia due to the transient hypotension induced by LPE. GAL (1-29) did not induce any statistical changes in the slopes compared with the controls at any dose tested. However, i.v. injection of 0.1 nmol of GAL (1-15) produces a decrease of BRR sensitivity (p<0.02) when compared with the control of the GAL (1-29) groups (p<0.05). The blocking effect of BBR by GAL (1-15) might explain in part its vasopressor action and may indicate a major role for this galanin fragment in central cardiovascular regulation could be suggested possibly acting on specific receptor subtypes for N-terminals fragments of galanin.

This work was supported by the Swedish MRC (0X4-715).
554.13

We used an in-vitro approach to investigate the influence of opiates and melanotropin on the activity of noradrenergic neurons in the caudal medulla of the rat. [N-Me-D-Phe]-MSH (NDF-MSH) had no effect on the synaptic activity of noradrenergic neurons in the A2 as gauged by in-vitro L-DOPA accumulation and dopamine release (DDM) in explants. NDF-MSH reduced the noradrenaline (NE) content of explants treated with N5D-1015 but had no effect on basal or 


554.14
PRESYNAPTIC DELIVERY OF CGRP BY TRANSFECTED PC12 CELL CO-CULTURES. F. S. Schweitzer*, C.-J. Jing, and Susan J. H. Teo-Cheng*. Dept. of Anatomy & Cell Biology, UCLA Medical School, Los Angeles, CA 90024 and **Laboratory of Neurobiology, NINDS, NIH, Bethesda, MD 20892.

In order to examine the role of CGRP in synapse formation and maintenance, we have engineered PC12 cells to synthesize and secrete CGRP from regulated (presynaptic) secretory vesicles. We have previously shown that the transient expression of a full-length CGRP with norepinephrine-containing vesicles on density gradient fractionation, and is secreted upon depolarization of the PC12 cells in the presence of calcium. We have shown by immunoelectron microscopy that the CGRP is contained exclusively in dense-core vesicles in both undifferentiated and NGF-treated PC12 cells. CGRP is absent from the synaptophysin- and SV-2r rich small clear vesicles. The CGRP in the dense-core vesicles is therefore presumably the source of the CGRP secreted from the cells upon stimulation.

We have now co-cultured wild-type and CGRP-expressing PC12 cells with C2 myotubes, to examine the effect of the CGRP on the induction of ACh receptors and the formation of ACh receptor clusters. Preliminary evidence indicates that the location of the ACh receptor clusters on the surface of the myotubes is correlated with the presence of CGRP. We are now examining these co-cultures for evidence of a specific effect of CGRP on the rate or extent of ACh receptor clustering.

554.15
Pancreastatin Stimulates Cobalt Uptake by Hippocampal Neurons. C. S. Toonum* W. R. Millington. Division of Molecular Biology and Biochemistry, Univ. of Missouri-Kansas City, Kansas City, MO 64108.

Pancreastatin, a 49 amino acid peptide derived from chromogranin A, is widely distributed in neural and endocrine tissues. Pancreastatin modulates the secretion and synthesis of various peptides; however, the evidence of its function in brain exists to date. We tested whether pancreastatin activates hippocampal neurons by using a biochemical assay which measures cobalt uptake through a calcium permeable channel in tissue slices (Nakan et al. Neurosci 7590, 1991). Our initial experiments confirmed that kainate, an agonist receptor antagonist, produced a dose-dependent accumulation (1-100 μM) of cobalt in hippocampal neurons of the dentate gyrus and Ammon's horn (CA). Coincubation with the glutamate agonist, kynurenic (5mM) blocked the response completely. Pancreastatin also produced a concentration related activation of cobalt uptake, albeit at considerably lower concentrations (0.5, 1 or 10 μM) than kainate. The regional distributions of pancreastatin and kainate responsive neurons were similar. Pancreastatin (500μM) stimulated cobalt uptake in areas CA1 and CA2, whereas higher concentrations (1-100mM) also promote cobalt uptake into CA3 and pyramidal and hilar cells of the dentate gyrus. At a 2 μM concentration, cobalt uptake was visible only in small portions of the dentrites, whereas, at higher concentrations, the cell bodies were also labelled. Interestingly, pancreastatin stimulated cobalt uptake was also blocked by kynurenic (100μM) suggesting that a glutamate receptor may be involved in the response. These data demonstrate that pancreastatin activates hippocampal neurons, suggesting that it may function as a neurotransmitter. Supported by the Scientific Education Partnership of the Marion Merrell Dow Foundation.

554.17
ENDOTHELIN-1 ACTIVATES CHEMOSENSORY TYPE I CELLS AND POTENTIATES THE HYPOXIC RESPONSE IN RABBIT CAROTID BODY. J. Chen, I. He, B. Dinger and S.J. Dignam*. Dept. of Physiol. Univ. of Utah Sch. of Med., Salt Lake City, UT 84108

Endothelin (ET) and ET mRNA have been localized to diverse structures in the central and peripheral nervous systems, and ET has been demonstrated to evoke release of catecholamines (CA) from adrenal chromaffin cells and corpus striata. In many tissues, the effects of ET are mediated by G-protein coupled receptors which initiate the hydrolysis of phosphatidylinositol. In the present study, we have examined the effects of ET-1 on insulin-phosphate (IP3) formation, CA release and carotid sinuses nerve activity elicited from in vitro superfused rabbit carotid bodies where vascular effects are eliminated. Submicromolar and micromolar concentrations (0,01, 0,1, 0,5 and 1 μM) of ET-1 elevated the accumulated IP3 (p<0,01), respectively. Similar concentrations (0,1 and 1 μM) of ET-1 potentiated the hypoxic-evoked release of CA by 1,36- and 2,16-fold (p<0,05), respectively. Finally, CSN activity evoked by hypoxic superfusion solutions (equilibrated with 20% O2) was elevated by 21,32% ± 5,84% (X ± SEM, p<0,001) in the presence of 1 μM ET. In preliminary experiments, ET-3 appeared to be less potent with respect to IP3 formation, CA release and CSN discharge. The data suggest that ET actions are mediated by elevated intracellular Ca2+ concentrations and protein kinase C in chemosensory type I cells. Endothelin may act as physiological antagonists to atrial natriuretic peptide, which are endogenous to the carotid body and exert powerful inhibitory influences on the carotid chemoreceptor. Supported by USPHS grants NS12636 and NS07938.

554.18

SDFNPLFlamamide (PF2), originally isolated from the free-living nematode P. redivivus, induces profound flecainide paralysis in neumuscular segments of the parasitic nematode, Ascaris suum, at 1-10 μM. We conducted binding studies to characterize the receptor for this peptide using [3H]PF1 (134 Ci/mmol) and membrane preparations from P. redivivus (whole nerve) and Ascaris (muscle, hypodermis, ovaries). Among the Ascaris tissues, preparations, a low level of specific binding was detectable only in the hypodermal membranes. Backwash analysis of the [3H]PF2 binding to P. redivivus membranes indicated both a high and low affinity binding site for the peptide. The high affinity site is characterized by a Kd of 3.7 nM and Bmax of 8.5 fmol/mg protein. Kd and Bmax values of 23.5 nM and 58.3 fmol/mg protein, respectively, were obtained for the low affinity site. Specific binding in these studies accounted for 49.1% of the total bound, and total binding was 1% of the label added. Competition binding studies revealed that, in the P. redivivus membrane preparation, the affinity of PF2 for SDFNPLFlamamide (PF2), another neuropeptide isolated from P. redivivus that has inhibitory effects on A. suum muscle.
554.20 STRUCTURE-ACTIVITY RELATIONSHIPS OF NEMATODE FMRFamide-like Neuropeptides in ASCARIS SUUM
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We examined the structure-activity relationships of two FMRFamide-like neuropeptides found in nematode, AF1 (KNEFIRFamide) and PF1 (SDPNFLRamide). We substituted alanine for each amino acid to generate a series of analogs that could pinpoint residues important for biological activity. We also tested a series of PF1 analogs in which each amino acid was replaced with its d-isomer. Truncated AF1 and PF1 analogs were similarly analyzed. Activity was measured in an Ascaris suum muscle strip preparation. For both AF1 and PF1, elimination of the C-terminal amide abolished activity. In the AF1 series, N-terminal deletions or extensions had unfavorable effects on potency. 

555.4 EFFECTS OF QUINOLINIC ACID LESIONS ON THE DISTRIBUTION OF D3 RECEPTORS IN RAT BRAIN
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Quinolinic acid lesions of the cell bodies in the nucleus accumbens (NAC) were used to examine the cellular localization of D3 receptors in the rat brain. A unilateral injection of quinolinic acid (150 nmol/0.5 μl) was administered into the NAC (ML+1.5; AP+1.5; DV-6.8 from the dura) at a rate of 0.1 μl/min. The contralateral NAC received a vehicle injection. Eight days later, the animals were sacrificed and coronal sections were processed for histology and quantitative autoradiography. D3 and D2 receptors were labelled with [125I]-Trans-OH-PIPAT and [125I]-NCQ-298, respectively. A marked loss of neuronal perikarya was observed in the shell and core of the NAC surrounding the site of injection. The density of D3 receptors was reduced in both the shell (dorsal - 60%; ventral - 43±4%) and the core (dorsal - 54±10%; ventral - 42±6%) of the NAC. Quinolinic acid has been shown to eliminate GABAergic effenter neurons at this dose, suggesting that D3 receptors are on the cell bodies or dendrites of these cells. The density of D2 receptors in the NAC was not altered by the lesion, indicating that D2 and D3 receptors are not co-localized on these cells. The density of D3 receptors was also reduced in the (ipsilateral substantia nigra pars compacta (45±11%) and the ventral tegmental area. This result suggests that D3 receptors are also located on the terminals of striatogenous fibers originating in the NAC. The D1 receptor has a similar cell body and terminal localization, suggesting that D1 and D3 terminal receptors may be co-expressed by some cells in the NAC. (Supported by USPHS GM 34781 and NS-24538)

CATECHOLAMINE RECEPTORS VI

555.3 EFFECTS OF PRENATAL IV COCAINE AND GENDER ON D2 AND D3 RECEPTORS IN THE NUCLEUS ACCUMBENS, D.W. Wallace*, C.P. MacIntosh and R.M. Booth, Dept. of Pharmacology, College of Medicine and College of Pharmacy, University of Kentucky, Lexington, KY 40506.
The effect of prenatal cocaine treatment on the density of D2 and D3 receptors was examined in the striatum and nucleus accumbens of male and female offspring. Intravenous cocaine injections were given on gestational days 6-14 (3 mg/kg X 1 daily) and on postnatal days 15-20 (3 mg/kg X 2 daily). MacIntosh et al., (1994). Pups (Sprague-Dawley) were sacrificed on postnatal day 35 and striatum/nucleus accumbens tissue removed and frozen (tissue homogenates) or whole brains were blocked and frozen (neurochemistry). Binding analysis of D2 and D3 receptor density was performed with [3H]-(-)-6-OH-DA (5 nM) and D2 receptor density was determined by [3H]-(+)-6-OH-DA (5 nM). All analyses were carried out at room temperature (21±1°C) for 90 minutes and terminated either by rapid filtration (homogenates) or by two successive 2 minute washes in ice-cold buffer (autoradiography). Densities of both D2 and D3 receptors, as determined by quantitative densitometry, were based on appropriate standards which were co-exposed with tissue sections. 

We examined the effects of alcohol consumption on the density of dopamine D1 receptors in the rostral and caudal striatum, nucleus accumbens, caudal ventral, frontal cortex, ventral pallidum and globus pallidus of Fischer 344 rats. In Fischer 344 rats, 23% of 28390 was used to radiolabel dopamine D1 receptors on the rostral striatum brain section. An improved binding technique was obtained from the 5, 14 and 24 month Fischer 344 rats that were per-fed a control or ethanol-containing liquid diet on a chronic basis prior to sacrifice. Additional rats received the control diet or ethanol diet for 28 days. FMRFamide-like neuropeptides were used to label dopamine D2 receptors on twenty micron brain sections. Binding was determined using the NIH Image program.

The results of these experiments demonstrated an age-related decrease in dopamine D1 receptors in both the rostral and caudal striatum and in the frontal cortex. This age-related decline was typically found in both control and ethanol-fed rats. In control rats, binding was significantly decreased in the ventral pallidum of ethanol-fed rats. No significant differences were found in the Kd for [3H]-SCH 23390 binding.

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555.5 REPEATED CATECHOLAMINE ADMINISTRATION INCREASES DOPAMINE RECEPTOR-REGULATED ADENYL CYCLASE ACTIVITY IN RAT CAUDATE PUTAMEN. E.M. Unterwald*, J. Fillmore, and M.J. Kreek. The Rockefeller University, New York, NY 10021.

Cocaine binds to the dopamine transporter and prevents the release of dopamine into presynaptic dopaminergic terminals. Repeated daily cocaine administration to rats for 14 days has been shown to upregulate dopamine D2 receptors (Unsworth et al., 1994). Adenylyl cyclase activity was measured in homogenates of regions containing dopaminergic innervation using a method that measures cyclic AMP (cAMP) formed from ATP when the preparation is stimulated with dopamine receptor agonists. Male Fischer rats were injected three times daily at one-hour intervals with saline or cocaine HCl (15 mg/kg, ip) for 1, 7, or 14 days. The ability of dopamine and quinpirole to stimulate adenylyl cyclase in the rostral portion of the caudate putamen was examined using a CAMP radioisotope binding assay in crude membrane preparations. D2 agonists (-10^4 to -10^3 M) produced a dose-dependent increase in CAMP formation with a maximum stimulation of 207 ± 2.7% over basal levels in saline-injected rats. Administration of cocaine did not significantly affect dopamine-stimulated cyclase activity. SKF-82958, 10^4 to 10^3 M, produced a dose-dependent increase in CAMP formation with a maximum stimulation of 192 ± 5.9% over basal levels. Administration of cocaine for 1 or 7 days did not significantly alter SKF-82958-stimulated cyclase activity. However, 14 days of cocaine administration significantly increased the stimulation of cyclase activity by SKF-82958. These findings suggest that chronic, repeated cocaine administration results in an enhancement of D2 receptor-mediated effector function in the rostral caudate putamen. (Supported by grants from NIDA (EMU) and the Arrow Diamond Foundation (MKK)).

555.6 DOPAMINE-INDUCED DEPOLARIZATION OF MEDIAL Prefrontal CORtical NeURONS. W.-X. Shi* and B.S. Bunney, Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Dopamine (D) innervation in the prefrontal cortex (PFC) is particularly sensitive to stress and has a powerful influence on subcortical DA systems. Malfunction of this system has been suggested to play a critical role in the pathogenesis of schizophrenia. To this end, we may ask the question: what is the PFC? The PFC, as defined as a whole cell recordings from rat medial PFC neurons in an in vitro slice preparation. Confirming the result of a previous intracellular study (Pertson-Sonta et al., Brain Research, 1987), we find that DA is depolarized (1-200 µM) in mPFC neurons located in layers V and VI (5043, ranging from 2.5 to 15 mV). This effect of DA persisted in the medium containing TTX (1 µg/ml, x 30 mV and 3 mm Mg, 20 mm Mg), suggesting a direct effect of DA on the recorded cell. Using electrodes containing Lucite Yellow (0.1%), nine DA-responding neurons were intracellularly labeled, six of them were identified as pyramidal neurons and three as non-pyramidal neurons, suggesting that DA affects both projection and interneurons in the PFC. Surprisingly, however, the depolarizing effect of DA was not mimicked by either the D2 agonist SKF38393 [(1-10 µM)] or the D2 agonist quinpirole (1-10 µM) or by the mixture of the two (5:1). The mixed D2/D2 agonist apomorphine (5 µM) also produced no effect on PFC neurons (n=5). Furthermore, the D2 antagonist SCH23393 (10-30 µM, n=7) nor the D2 antagonist eticlopride (10 µM) blocked the effect of DA. None of these preliminary results suggest that the depolarizing effect of DA seen in PFC neurons is not mediated by the known DA receptors that should be sensitive to the DA active agents used above. Whether DA produces this effect by acting on a new type of receptor or non-specifically on other transmitter systems is not yet known. This work was supported by HHS award MH82849, the NPF, the NARSAD, and the State of Connecticut.

555.7 EXPRESSION OF D, RECEPTOR-MEDIATED INHIBITION OF MIDBRAIN DA NEURONS REQUIRES CO-ACTIVATION OF POSTSYNAPTIC D2 RECEPTORS. D.L. Smith, W.X. Shi, B.S. Bunney, Department of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

We previously reported that low doses of the D2 antagonist SCH23393 partially but significantly reversed dopamine-induced inhibition of substantia nigra dopamine (DA) neurons in paralyzed rats (low cervical level preparation) and suggested that D2 receptors are involved in the feedback control of DA neurons. However, when we determined that the D2 agonist SKF38393 alone had no consistent effect on DA cells. Since dopamine by releasing DA activates both D2 and D3 receptors and SKF38393 acts on only D2 receptors, we hypothesized that the expression of the mediated effect on DA neurons requires co-activation of D2 receptors. To test this hypothesis, we examined the effect of SKF38393 in animals pretreated with the D2 agonist quinpirole. Quinpirole potently inhibited DA neurons. To avoid the possibility that low doses of quinpirole (400 µg/kg, i.v.) were first used to produce a partial inhibition. In most of these cells (89%), however, SKF38393 (20 mg/kg, i.p.) produced no effect on the remaining activity of the cell. Considering the fact that D2 receptors in the substantia nigra are much more sensitive to a D2 agonist than to postsynaptic DA receptors, we further postulated that a D2-mediated effect depends on activation of postsynaptic D2 receptors and that the doses of quinpirole used were not high enough to achieve these receptors. To examine this possibility, we injected quinpirole slowly to desensitize DA autoreceptors. By doing this, we were able to inject 40 µg/kg or more of quinpirole while keeping the cell active. In most of these cells (89%), SKF38393 clearly produced a further inhibition of firing, which could be completely reversed by SCH23393 (20-40 µg/kg, i.v.). These results suggest that activation of D2 receptors has an inhibitory effect on DA neurons and the expression of this effect requires co-activation of D3 receptors most likely located on non-DA neurons. Supported by MH82849, the NPF, the NARSAD, and the State of Connecticut.

555.8 THE D2 RECEPTOR AGONIST QUINPIROLE EXCITES NEURONS OF THE GLOBUS PALLIDUS IN FREELY-MOVING RATS. K.C. Hooper, D.A. Banks, and G.V. Rebec. Program in Neural Science and Dept. of Psychology, Indiana University, Bloomington, IN 47405.

We have previously shown that selective stimulation of D2 receptors by systemic administration of the D2 agonist quinpirole (LY-171555) inhibits the activity of neurons in the striatum of awake, behaving rats (Hooper et al., Soci Neurosci. Abstr. 1992; 18:995).

To assess how this change in striatal activity affects neuronal activity in the globus pallidus, an output nucleus of the basal ganglia that receives an inhibitory projection from the striatum, we monitored single-unit activity in the globus pallidus of freely-moving male rats in response to the systemic administration of quinpirole at a dose known to inhibit striatal activity (1.0 mg/kg). Our results indicate that quinpirole excites neurones in the globus pallidus of awake rats. This effect probably occurs through a disinhibitory mechanism since GABAergic striatal cells, which project to the globus pallidus, are inhibited by quinpirole. Consistent with this view, preliminary data indicate that the quinpirole-induced excitation of pallidal neurones is reversed by eticlopride, a D2 receptor antagonist. Supported by USPHS Grant DA 02451.
555.11

SELECTIVE DOPAMINE D3 RECEPTOR ACTIVATION BY 7-OH-DPAT INDUCES HYPOTHYROIDISM AND DECREASES STRIATAL DOPAMINE RELEASE BUT NOT METABOLISM IN ANKLE RATS. R.G. Gross, P.J. Gershon, J. Dostrovsky, J. Gershon, and M.E. Liebman.

We have previously found a rather good correlation (r=0.75, p<0.02) between the abilities of acute typical and atypical antipsychotics to preferentially dopamine (DA) metabolism or release in the rat dorsal striatum in vivo and their relative potencies at D2 and D3 receptor. Present microdialysis study in awake Wistar rats with se.

le mide of DA agonists 7-OH-DPAT (0.57 - 0.8 mg/kg, ICV) and (-)-7-OH-DPAT (0.0005 - 0.04) were used to estimate the interstitial free concentration (ITC) of 7-OH-DPAT in the dorsal striatum. 7-OH-DPAT as well as DA, DOPAC and HVA were detected by HPLC in 20 minutes after i.p. administration of 7-OH-DPAT in doses ranging from 1 to 10 mg/kg. The results were as follows:

The maximum concentration of 7-OH-DPAT was reached at 18-29 min (n=4). The marked decrease of DA release (to 20 - 30% of basal) and metabolism (to 50%) was also observed. Due to approximately a linear relation between the dose - dopamine concentration observed it was inferred that at 7-OH-DPAT dose of 0.55 mg/kg ITC which would not exceed 16 nM. Importantly, 7-OH-DPAT in this dose produced significant decrease of DA release (to about 80%), but failed to affect metabolites level in the rat striatum, whereas in dose 0.25 mg/kg (calculated ITC 80 nm) both DA release and metabolism were approximately of 30 and 70%, respectively. The threshold dose for the significant decrease of DA release was ~0.005 mg/kg (calculated ITC 8 nm), while even in a dose 0.01 mg/kg (calculated ITC 3.2 nm) the drug induced hypothyroidism. In conclusion, 7-OH-DPAT at the dose range well below the affinity to D2 receptor induces hypothyroidism and decreases DA release but not its metabolism in the dorsal striatum of awake rats.

555.12


Dopaminergic autoreceptors control ascending dopaminergic pathways Here, we employed the novel, selective D3 antagonist, S 14297, to evaluate the potential contribution of D3 receptors to this action. As a measure of synthesis, the ratio of the levels of the dopaminergic metabolite, DOPAC in the caudate versus 2.8-fold lower than in 7-OH-DPAT (0.016) at DA release, and therefore, failed to modify levels of DA. S 14297 (20.0) was inactive. In conclusion, D3 autoreceptors inhibit synthesis and release of DA in ascending dopaminergic projections and S 14297 acts as an antagonist at these sites. These data suggest a role of D3 receptors in the modulation of mood and in the pathogenesis of psychiatric and motor disorders.

555.13


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Behavioral experiments in rats show that the D3 preferring-agonists, SKF 38393 and SCH 23390 were more efficacious in reducing exploratory activity as compared to D2 agonists. In the same animals, the accumulation of DOPA or the release of dopamine in various brain regions was not affected by the ED50 dose causing reducing exploratory activity. In contrast, for the more D2 preferring-agonists, such as apomorphine, (+)-3PPP and quinpirole, there was a closer correlation between doses that reduce locomotor activity and doses that reduce DOPA accumulation and dopamine release. A similar trend was also observed for the D2 selective compound U-91356. The data suggest that the D2 selective agonists, in contrast to the more D2 selective agonists, are able to reduce locomotor activity at doses that do not affect dopamine release and/or synthesis. Furthermore, these data are in line with the hypothesis that the D2 receptor is postsynaptic with an inhibitory influence on rat locomotor activity. Our present work focuses on the interaction between D2 preferring-agonists and antagonists on behavioral and neurochemical parameters in order to shed further light on the possible functional role of the D2 receptor.

555.15


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In studies with cloned subtypes of the D2 receptor subfamily, Pramipexole (PPX) has higher affinity for the D3 as compared to the D2 and D3 subtypes, and none for D2 receptors (Neurovisc Abs 1980, 93). Using Sosoloff's quantitative 2-deoxyglucose (2DG) autoradiography method (J Neurochem 28:897, 1980). Using this method in several basal ganglia regions (substantia nigra, subthalamic nucleus), but increased it even more in sensory and motor cortex. No significant effects were observed in microdialysis DA areas (n. accumbens, olfactory tubercles, lateral septum and CA1 of the hippocampus), but the data was not conclusive in these areas. In the caudate nucleus, a D2 postsynaptic area high in D2 receptors, but in which D3 pharmacological effects also occur (Neurovisc Abs 1980, 93). 18.4 PPX binding sites were found in the lateral amygdala, an area dominated by D2 receptors (PAN 89:1855, 1992). Using D2 agonists i.c.v. in the n. accumbens, olfactory tubercle, as well as in the caudate nucleus, a D2 postsynaptic area high in D2 receptors, but in which D3 pharmacological effects also occur (Neurovisc Abs 1980, 93). 1.4 PPX binding sites were found in the VTA, an area rich in DA cell bodies, and in sensory and motor cortex. Thus, increases in energy metabolism occurred in areas low in D3 binding sites, but not in areas high in D3 binding sites, i.e., substantia nigra, subthalamic nucleus. Therefore, the ability of D2 DG autoradiography to measure downstream functional effects of pharmacological agents.

555.16


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The dopamine D3 selective agonist R- (+)-7-OH-DPAT produced a significant increase in reward thresholds over a wide dose range (0.094 - 96.0 nmol/kg, s.c.) similar to the well described effects of the classical D2 antagonist haloperidol (0.005 - 0.16 mg/kg, i.p.) when tested in rats trained to self-stimulate in a rate-intensity brain stimulation reward paradigm. At higher doses, 7-OH-DPAT produced a significant facilitation of reward thresholds.

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CHARACTERIZATION OF A SELECTIVE DOPAMINE 3 RECEPTOR AGONIST LIGAND, (H)[^3H]PD 128907. H. C. Akam and T.C. Tsai. Jr. Department of Pharmacology, University of Rochester Medical Center, Rochester, NY.

To determine the characterization of a novel dopamine (D3) receptor agonist, we measured the binding of (^3H)PD 128907 to brain regions from naive rats. The binding was saturable and specific. The KD was 2.1 nM, and the Bmax was 430 fmol/mg protein. The D3 receptor binding was highest in the striatum and lowest in the cerebral cortex. The results suggest that (^3H)PD 128907 can be used as a tool to study the D3 receptor in vivo.


PD 128907 has been shown to be a dopamine (D3) receptor agonist-like compound. The authors used autoradiography to study the effects of PD 128907 on DA3 receptors in the rat brain. They found that PD 128907 increased DA3 receptor binding in the striatum, hippocampus, and hypothalamus. The authors suggest that these results may have implications for the treatment of Parkinson's disease.

REGIONAL DISTRIBUTION OF DOPAMINE receptors in the brain. H.C. Akam and T.C. Tsai. Jr. Department of Pharmacology, University of Rochester Medical Center, Rochester, NY.

The authors measured the distribution of D1 and D2 receptors in the rat brain using in vivo autoradiography. They found that D1 receptors were highest in the striatum and lowest in the thalamus, while D2 receptors were highest in the caudate-putamen and lowest in the hippocampus. These results suggest that D1 and D2 receptors may have different functions in the brain.

CHARACTERIZATION OF A NEW HIGHPERSEPTIC DOPAMINE 3 RECEPTOR AGONIST: (H)[^3H]PD 128907. H.C. Akam and T.C. Tsai. Jr. Department of Pharmacology, University of Rochester Medical Center, Rochester, NY.

The authors characterized a new dopamine (D3) receptor agonist, (^3H)PD 128907. They found that the KD was 2.1 nM, and the Bmax was 430 fmol/mg protein. The D3 receptor binding was highest in the striatum and lowest in the cerebral cortex. These results suggest that (^3H)PD 128907 can be used as a tool to study the D3 receptor in vivo.
556.1 INCREASED NITRIC OXIDE SYNTHASE ACTIVITY BY SIN-1 AND SODIUM NITROPRUSSIDE IN INTACT NEURAL CELLS. J. H. and P. E. El-Fakahany, Division of Neuroscience Research in Psychiatry, University of Minnesota Medical School, Minneapolis, MN 55455.

It has been shown that nitric oxide (NO) regulates NO synthase activity through negative feedback in cytosolic enzyme preparations in a variety of cell types. We tested the effects of the NO-generating compounds 5'-diamino-N-acetylornithine (SNAP), 5'-diaminopentane (SNP) and sodium nitroprusside (SNP) on NO synthase activity in intact neuramiblastoma NIE-115 cells. SIN-1 and SNP at concentrations which elicited almost complete inhibition of NO synthase activity in cytosolic enzyme preparations of these cells only inhibited about 30% of the enzyme activity in intact cells, as measured by the conversion of [3H]-arginine to [3H]-citrulline during incubation for 45 min. Surprisingly, SIN-1 and SNP stimulated [3H]-citrulline formation when cells were incubated with the compounds for > 1.5 hours. Neither inhibitor nor stimulatory effects of SNAP on NO synthase were observed in the intact NIE-115 cells. This is in contrast to the inhibitory effects of SNAP in cytosolic preparations of the enzyme. Measurement of the activity of lactate dehydrogenase indicated that there was no parallel increase in cell permeability in response to SIN-1 or SNP. The stimulation by SIN-1 and SNP of NO synthase activity was completely blocked by 3 mM EGTA, suggesting that an influx of Ca2+ is activated by the compounds in intact NIE-115 cells. Moreover, hemoglobin, superoxide dismutase, defereroxamine attenuated the effects of SIN-1, but not of SNP, on [3H]-citrulline formation. Our results demonstrate that NO-generating compounds might somehow enhance Ca2+ influx, resulting in an up-regulation of NO synthase activity. These effects might be dependent on the chemical nature of the NO species released by the compounds.

556.3 SIMPLE GUANIDINE ANTAGONISTS OF NITRIC OXIDE SYNTHASE. J. F. Macdonald, D. W. Reif, A. Wallace, Fisons Pharmaceuticals Divisional Research and Development, PO Box 1710, Rochester NY 14603.

The role of nitric oxide synthase (NOS) in the function of the nervous system under normal and ischemia/reperfusion conditions is a topic of great interest. The current known antagonists of NOS are limited in that they lack selectivity between the brain, endothelium and macrophage NOS isoforms. Thus, selective antagonists would be useful tools to elucidate the role of the three NOS isoforms. Using the H3-arginine to H3-citrulline conversion assay (PNAS 87, 682-685, (1990)), compounds were assayed for inhibition of rat cerebellar , mouse macrophage, and human endothelial NOS. In our initial search for novel antagonists of NOS, we speculated that simple guanidines would be a reasonable starting point. The screening results for a series of substituted guanidines will be presented. Briefly, we have discovered small simple guanidines which inhibit the NOSs in the micromolar range (similar to methylarginine and nitroarginine), but are not selective versus a particular isoform. We will present results which will demonstrate that all of the NOS isoforms are inhibited by small cyclic guanidines and that inhibition is very dependent upon the size of the molecule.

556.5 PHOTONEURAL REGULATION OF RAT PINEAL NITRIC OXIDE SYNTHASE. N.C. Schub*, Jiri Vasicek and P.E. Schulz Div of Clinical Psychopharmacology, Dept of Psychiatry of the University of Geneva, 1225 Cbde-Bourg, Switzerland.

Nitric oxide (NO) appears to play a role in the regulation of cGMP synthesis in the rat pineal gland. Pineal nitric oxide synthase (NOS) is dependent on calcium for its activity, and all its biochemical characteristics are similar to the constitutive form of the enzyme present in the cerebrovasculature. The factors regulating the expression of the constitutive form of NOS in the central nervous system remain largely unknown. We report here a photoneural regulation of NOS activity in the rat pineal gland. In the absence of the adrenergic stimulation following light exposure (LL) or denervation (bilaterial superior cervical gangliectomy), pineal NOS activity is markedly reduced. A maximal drop is measured after 8 days in LL (< 80% versus control). When rats are housed back in normal light-dark conditions (LD 12-12), pineal NOS activity returns to normal after 4 days. A partial decrease in pineal NOS activity is also observed when rats are placed for 8 days in LD 18:6 or shorter dark phases indicating that pineal NOS activity reflects the length of the dark phase. Because it is known that norepinephrine (NE) is released at night from the nerve endings in the pineal gland and this release is blocked by exposure to light, our data suggest that NOS is controlled by adrenergic mechanisms. Our observation may also explain the lack of cyclic GMP response to NE released in animals housed in constant light.

556.6 TRANSMITTER MODULATION OF NMDA-ACTIVATED NITRIC OXIDE SYNTHASE (NOS) ACTIVITY IN RAT FRONTAL CORTEX. S. Aleksargy* and K. M. Johnson. The Department of Pharmacology and Toxicology, The University of Texas Medical Branch, Galveston, TX 77555-1031.

We have previously reported that activation of classical N-methyl-D-aspartate (NMDA) receptors can stimulate NOS activity in the frontal cortex. In this study we focused on other transmitter that may activate NOS or modulate the NMDA-activation of NOS. We found that of the transmitters examined, glutamate and acetylcholine (ACh) receptor agonists were the only ones to affect NOS activity. Glutamate stimulated NOS activity and this response is blocked by NMDA antagonists, but was unaffected by non-NMDA antagonists. These data are substantiated by the fact that AMPA, kainic acid (KA) and quisqualic acid (QX) did not increase NOS activity, even in the presence of dizoxide which prevents desensitization of the glutamate receptors, suggesting that the glutamate response was due to activation of the NMDA receptor in this preparation.

Norepinephrine, dopamine and y-aminobutyric acid (GABA) had no effect on either the basal or NMDA-activated NOS. However, while the basal NOS activity was unaffected by ACh agonists, 1 mM carbamylcholine chloride, a mixed ACh agonist, inhibited the NMDA response completely. This inhibition was not reversed by atropine, nor was it mimicked by muscaricine. Nicotine (30 μM) inhibited the NMDA response, but this inhibition was not complete, perhaps due to receptor desensitization. The inhibitory effect of nicotine was insensitive to hemicholinum and duspiperazine. Complete pharmacological characterization of this apparent novel nicotinic mechanism is currently under investigation. Supported by DA-02073.


The presence of NADPH-dihydrogenase-positive nerves in the rat uterus, and the role of nitric oxide (NO) in the pregnant animal, suggests that neuropeptides containing NO may be important in regulating myometrial activity, uterine blood flow and other reproductive functions. NOS was isolated from the uterus of adult female rats by diethylaminoethyl (DEAE) column and further purified by HPLC agarose. The chromatographic properties revealed two bands, myosin and NOS. The molecular weights of both isoforms were approximately 155 Kd by SDS PAGE which was similar to NO, and NO, from rat brain. The enzymes required NADPH, Ca2+ and calmodulin as cofactors. However, in the absence of calmodulin or other calcium NOP, was reduced by 96%, while NOS was reduced by 70%. This maximal enzyme activity was similar for brain. These results demonstrate that the rat uterus contains NOS and the fundamental kinetic constants, Km and Vmax, are similar to brain NOS. The uterine NOS is NADPH-dependent and different enzyme isoforms are suggested by the calmodulin-dependence and trifluoroperazine-dependence of both uterine and brain NOS.

556.8 INDUCIBLE NITRIC OXIDE SYNTHASE IN CA1 NEURONS. L. Divac* and J. Beisner, Department of Medical Physiology, University of Copenhagen, Denmark, and Department of Morphology, University of Las Palmas de Gran Canaria, Spain.

Our results, obtained in collaboration with several colleagues, have shown that NAPDH-diaphorase activity can be induced in the pyramidal CA1 neurons of the hippocampus by different agents. The effective agents are: electrical stimulation, mild stress, and direct injury of the hippocampus, either in vitro by slicing or in vivo by surgical injections. After the induction, we found in hippocampal tissue an increased formation of cyclic GMP, indicating that the induced molecule has NOS enzymatic function. The induction of NAPDH-diaphorase activity in CA1 neurons following intrahippocampal injections of saline can be abolished by pretreatment with anti-inflammatory drugs, dexamethasone or indomethacin, but not by a NOS inhibitor, L-NAME. Surprisingly, an antibody raised against the constitutive neuronal NOS did not stain the CA1 neurons which are demonstrably stained for NAPDH-diaphorase. We conclude tentatively that a molecule, different from the constitutive NOS with both NAPDH-diaphorase and NOS activities, can be induced in some neurons.
556.7 DISTRIBUTION OF NITRIC OXIDE SYNTHASE (NOS) IN RAT CENTRAL NERVOUS SYSTEM (CNS): A COMPARATIVE STUDY OF QUANTITATIVE AUTORADIOGRAPHY AND ENZYME ACTIVITY


Departments of Anesthesiology (S.M., P.F.) Neurology and Anatomy/Cell Biology (O.R.H.), Mount Sinai Medical Center, New York, NY 10029.

NOS is a ubiquitous enzyme in the mammalian CNS. However, a meaningful assessment of its distribution is lacking. We have developed a novel method for quantitative autoradiography of 7H(-)nitric-oxide binding. Data obtained with this method have been compared to the distribution of enzyme activity across rat CNS measured by the cyclic assay. The (100 µM) nitroarginine-sensitive NOS activity was assayed (30°C, 10 min, 0.25-40 µM (H)-[14C]arginine) in three preparations: a crude homogenate and cytoplastic andparticles fractions derived from it. Quantitative autoradiography and nitroarginine binding were done under the same condition with 50 µM (H)-nitric-oxide, 20 µM cronal cytoplasmic sections of rat brain.

The values were compared to the enzyme across the rat CNS (1-3 µM) while the Vmax (fmol/mg of tissue) varied: the highest was observed in the cerebellum (000) and the lowest was found in the spinal cord (10). 20-40% of NOS activity was found in the particulate fraction, indicating an uneven distribution of the enzyme within cell compartments. Areas of highest binding included the olfactory tubercle, amygdala, fornix, subterritia nigra, periadendal gray, and the cerebellar cortex. Intermediate binding levels were obtained in the hypothalamus, hippocampus, septal nuclei, globus pallidus, and the superior colliculus. Low binding was observed in the frontal and parietal cortex, and the caudo-putamen. Arginine-sensitive (H)-nitric-oxide binding varied between 65% of total binding (olfactory tissue) to none (caudo-putamen).

This combination of two quantitative methods for assessing NOS is proposed as a powerful tool to study the enzyme in mammalian CNS.

556.8 LOCALIZATION AND BIOCHEMISTRY OF NADPH-DIPHORASE ACTIVITY IN THE DEVELOPING CHICK RETINA

R. E. Sigman and R. F. Smith

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NADPH-Diphorase (ND) activity, reported to be identical to NOS in the chick retina, has been found in specific groups of neurons in the CNS. We studied the expression of the enzyme in the developing chick retina. Enzyme activity was measured by spectrophotometric assays, using retinal homogenates with NADPH and nitrous blue tetrazolium. The immunohistochemistry, performed using incubating 4% paraformaldehyde-fixed cryostat sections, with a specific antibody raised in sheep, revealed the presence of stained nerve fibers and retinal pericytes (ER). An adult pattern was observed at E10, when photoreceptors and cones were present, and sensitive. ND was detected biochemically in homogenates of retina at E14 and E16 retinas when we observed a 50% stimulation by Ca2+ and 70% inhibition by L-NMMA. The stimulation by Ca2+ decreased at E17 and was insignificant in post-hatched retinas. Inhibition curves obtained with increasing concentrations of NADPH and L-NAME (1M) gramine (NOS) in the presence and absence of Ca2+ suggested the presence of NOS in the retina. In conclusion, the biochemical data and different isoforms of NOS in the retina. The results showed that the NO biochemical assay can be used as a sensitive method for studying NO activity and that different isoforms of NOS are expressed in populations of chick retinal cells during development. (Supported by CMG and RO1-NS-099).

556.9 NITRIC OXIDE SYNTASE ACTIVITY INCREASED IN BRAIN IN EXPERIMENTAL HEPATIC ENCEPHALOPATHY

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Glutamatergic synaptic dysfunction has been proposed as a causal factor in hepatic encephalopathy. Increased glutamate release (Butterworth et al., J. Neurochem. 66, 1481, 1991) and decreased glutamate uptake into NMDA receptors (Peterson et al., J. Neurochem. 55, 386, 1990) were previously reported in the brains of rats following portacaval anastomosis. Our results demonstrate that the activities of this enzyme were significantly increased in cerebral cortex (65%, p<0.05), cerebellum (54%, p<0.001), hippocampus (88%, p<0.001) and striatum (64%, p<0.001) of shunted rats compared to sham-operated controls. As the transport system is a prerequisite for nitric oxide production, we also studied [H]arginine transport into the cerebellar and cortical synaptosomes prepared from the brains of portacaval shunted and sham operated rats. [H]arginine uptake was significantly increased (45-55%, p<0.001) in both cortex and cerebellum. Alterations of the NOS system and the resultant increased production of nitric oxide and cGMP in brain could be of pathophysiologic significance in hepatic encephalopathy. (Supported by MRC Canada).

556.10 NITRIC OXIDE AND CYCLIC GMP ACTIVATE LOCUS COERULEUS NEURONS IN RAT BRAIN SLICES: ROLE OF CYCLIC GMP-DEPENDENT PROTEIN KINASE

J.H. Kopant., J. Procha and G.K. Aghajanian


Nitric oxide (NO) has recently been shown to be a diffusible second messenger capable of stimulating soluble guanylate cyclase. Since the noradrenergic locus coeruleus (LC) is rich in the α, β, and γ-subsunits of soluble guanylate cyclase (Funayama et al., Mol. Brain Res. 20, 1993) we investigated the role of NO/cyclic GMP pathway in the LC. Extracellular electrophysiological recordings demonstrated that bath-applied 8-bromo-cyclic GMP (8BrgcGMP, 1mM) caused a gradual excitation of LC neurons; a more rapid response occurred when cyclic GMP (100mM) was introduced via patch pipettes. Similarly, during extracellular injection of NO donors sodium nitroprusside (SNP, 1mM) and N-nitro-S-acetylpenicilliamine (SNAP, 1mM) excited LC neurons. The SNP excitation could be blocked by prior application of 8BrgcGMP indicating that SNP was acting through the NO/cyclic GMP pathway. Furthermore, H-8 (100mM), a protein kinase inhibitor with a relatively high affinity for cyclic GMP-dependent protein kinase (PKG), antagonized both the SNP and the 8BrgcGMP activation of LC neurons consistent with an action through PKG. These results suggest that there may be a physiological role for the NO/cyclic GMP pathway in the LC.

556.11 NITRIC OXIDE (NO) GAS ENHANCES TRITIATED NOREPINEPHRINE ([3H]NNE) RELEASE FROM RAT HIPPOCAMPAL SLICES

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Previous studies in our lab have shown that NO gas enhances N-methyl-D-aspartate (NMDA)-stimulated release of preloaded [3H]NNE from rat brain slices in a dose-dependent, oxygen-sensitive, and cyclic GMP-independent manner. In this study we have attempted to determine the mechanism for the enhancement of neurotransmitter release from NO gas. Aminoguanidine-enhanced transmitter release was not due to buffer acidification or generation of NO degradation products, since reducing buffer pH below 7.3 inhibited NMDA-stimulated [3H]NNE release and nitrate or nitrite ions (3-10mM) had no significant effect on release. Carbon monoxide (10-30mM), another diatomic gas with properties similar to NO including enhanced binding to guanylate cyclase, did not significantly affect release. NO was reported to be modulated by mono-A, ADP-ribosylation of cellular proteins, but ADP-ribosylation inhibitors nicotinamide (10mM-10mM) and luminol (1mM-1mM) did not diminish the enhancement of transmission seen with NO. The NE reuptake inhibitor desmethylimipramine inhibited nor blocked the effect of NO, suggesting that NO was not acting via inhibition or reversal of the NE transport. The metabolic inhibitors sodium cyanide, potassium cyanide (0.1mM), and 2,4-dinitrophenol (1mM) all dose-dependently enhanced NMDA-stimulated [3H]NNE release similar to NO. These results suggest that NO may enhance transmitter release by a direct effect on the calcium homeostasis. Supported by NIAAA A10898 and NIDA Training Grant DA 07027.

556.12 CHARACTERIZATION OF NITRIC OXIDE-INDUCED [3H]NOREPINEPHRINE RELEASE FROM HIPPOCAMPAL SLICES


We have previously reported that a long exposure to hydroxyamine, a nitric oxide (NO) generator, induced a tetradotoxin-insensitive, Ca2+-dependent release of [3H]norepinephrine ([3H]NNE) from rat hippocampal slices (Lourt et al., Eur. J. Pharmacol., 1992). These data suggested that NO induced the release of [3H]NNE directly from the noradrenergic terminals via exocytosis. However, we recently made observations which suggest the involvement of both NO-induced glutamate (Glu) release and non-classical [3H]NNE release via reverse NE transport. When we applied only a 10 min pulse of 100 µM hydroxyamine, which evoked a 10-fold increase in the [3H]NNE release, was reduced by 80% by 5µM tetradotoxin and 1mM kynurenic, a Na+-channel blocker and a non-selective nortopicglutamate receptor antagonist, respectively. Ten µM CNQX and 100 µM GYKI 52466, competitive and non-competitive AMPA/kainate receptor agonists, respectively, reduced the hydroxyamine response by about 50%. The NMDA receptor antagonists AP-5 and MK-801 reduced the hydroxyamine response only at concentrations which also inhibited high potassium and reverse NE transport. However, GY 19755, another NMDA receptor antagonist which had no effect on the [3H]NNE transport, did not influence the hydroxyamine-induced [3H]NNE efflux. Ten µM dextroamphetamine 10 µM d-amphetamine, potent inhibitors of noradrenergic uptake, reduced the hydroxyamine-evoked release from hippocampal slices by about 70%. 5-nitro-1-cyanoethyl, another NO generator (300 to 3,000 µM), stimulated the efflux of both [3H]NNE and [3H]Glu from hippocampal synaptosomes. These data suggest that moderate NO stimuli can evoke [3H]NNE efflux both directly from the noradrenergic terminals and indirectly via releasing glutamine. Supported by DA-02073 (K.M.J.) and the P. and A. Forman Research Foundation (G.L.).
LIPOLYSACCHARIDE INHIBITS THE PRODUCTION OF NITRIC OXIDE IN RAT PHEOCHROMOCYTA Cells. G. Cao*, R.L. Prior*, J.G. Strain and J.A. Joseph* *USDA- ARS, INRC at Tufts Univ. Boston, MA O2111 and *Dept. of Nutr. Sci., Univ. of Conn. Storrs, CT 06296 Nitric oxide (NO) is a recently recognized messenger molecule mediating diverse functions including vasodilation, neurotransmission, and antimicrobial and antitumor activities. NO is produced from l-arginine by NO synthase which has two isozymes, i.e. the constitutive NO synthase (cNOS) and inducible NO synthase (iNOS). The iNOS can be induced by bacterial lipopolysaccharide (LPS), phenol esters, and some cytokines like TNF-a, IFN-g and L-1B. However, the mechanism involved in the negative regulation of the NO production is not clear. Here, we report an inhibition of 43% and 58% in nitrite production in L10 cells, a variant of IC-12 rat pheochromocytoma cells, by adding 1 or 10 ug/ml LPS, respectively in the culture media for 3 days. Nitrite production was also inhibited by 43% in control cells not exposed to LPS but incubated with 0.5 mM N-methyl-l-arginine acetate, a compound that specifically and competitively inhibits NOS. The results suggested that these L10 cells were from cNOS and could be negatively regulated by LPS.

DOES CARBON MONOXIDE REGULATE CYCLIC GMP LEVELS IN THE RAT BRAIN IN VIVO? J.V. Laitinen* and K.E.M. Laitinen, Rovaniemi, Finland. RIA was used to study in vivo regulation of brain cGMP levels by the putative gaseous messengers nitric oxide (NO) and carbon monoxide (CO). Anesthetized rats were implanted with the microdialysis probes in the frontal cortex (FC) or the cerebellum. FC location was similar in both regions, but we observed marked differences in responses to the NO donor sodium nitroprusside (SNP) or to CO. Most notably, SNP was 3-fold in the FC and 90-fold in the CB. The perfusion with CO-saturated aCSF stimulated cortical cGMP levels transiently and reversibly but elicited an irreversible and delayed response in the CB. Perfusion with NO donor L-NMMA (2 mM) suppressed cerebellar cGMP by 75% but exhibited no potency in the FC. Quite opposite, local perfusion with the heme oxygenase (HO) inhibitor sin protoporphyrin IX (100 uM) suppressed basal cortical cGMP by 50% but was without effect in the FC, suggesting that CO, generated by HO activity, might regulate cortical cGMP production in vivo. As NOP may have actions independent of HO inhibition, we have begun to address these questions by using more specific tools including a microarray for HO activity in discrete brain regions, as well as antisense oligonucleotide targeting of NO gene in vivo. These tools are hoped to reveal whether endogenously produced CO participates in the maintenance of cortical cGMP levels in vivo.

REGULATION OF THE CAMP RESPONSE ELEMENT BINDING PROTEIN (CREB) IN THE LOCS COERULEUS (LC) AND NUCLEUS ACCUMBENS (NAc) BY DRUGS OF ABUSE. K.J. Whitney, D.S. Russell, D.W. Selt, M.D. Misriandranoo, G. Konrad*, S.E. Hyman* and E.F. Nestler. Laboratory of Molecular Psychiatry, Dept. of Psychiatry and Pharmacology, Yale School of Medicine, New Haven, CT 06510; *Massachusetts General Hospital, Charlestown, MA 02129. We have shown previously that chronic administration of morphine or cocaine alters the expression of the CREB-dependent gene and components of the CREB-interacting messenger system. These changes occur in specific brain regions known from behavioral and pharmacological studies to mediate aspects of drug action (see Neuron, 11:995, 1995). We are currently investigating the molecular mechanisms involved in these adaptations by focusing on regulation of CREB transcription factor (FCREB) in the NAc. Morphine administration regulates CREB phosphorylation in the LC, as would be expected given morphine regulation of the cAMP pathway (J. Neurochem, 51:1168, 1988). CREB protein was thought to be constitutively expressed in the brain, but we have found that chronic morphine increases levels of CREB immunoactivity in the LC. Interestingly, in the NAc, chronic morphine decreases CREB immunoreactivity. Preliminary evidence suggests that chronic cocaine also influences CREB levels in this brain region. In order to test whether CREB is directly involved in the observed changes in signal transduction proteins, we are investigating antisense PS oligonucleotides to CREB into the NAc. Chronic (5 day) antisense injections of CREB significantly decreased CREB in all regions examined, while CREB decrease in NAc appears to be associated with regulation of protein kinase A and G, as well as Fos-like proteins as would be expected. These studies should further elucidate the role of CREB in morphine and cocaine regulation of intracellular target proteins implicated in drug addiction.


Heme oxygenase (HO) catalyzes the oxidation of heme to biliverdin and carbon monoxide. Since HO is involved in the synthesis of prostaglandins, we have developed a new radioimmunoassay (RIA) for HO which uses 55Fe-labelled heme as substrate. As HO cleaves the heme ring to release Fe2+, this assay directly measures released 55Fe2+ bound to transferrin attached to Sepharose beads. As little as one pmol of 55Fe2+ can be readily detected, providing a sensitivity 50 to 100 times greater than conventional assays based on the absorbance of bilirubin formed from biliverdin by biliverdin reductase. The assay is specific, as no known brain enzyme other that HO releases Fe2+ from heme. The assay is simple to perform. Assays are conducted in the presence of the Sepharose-transferrin beads which are counted directly after incubation, centrifugation and washing. Thus, 100 to 200 assays can be performed in an hour. This assay permits detection of HO activity in very small brain samples and microwell samples of cell culture.

556.15 MORPHINE ATTENUATES FORSOKIN-INDUCED PREPROENKEPHALIN mRNA IN RAT STRIATUM: A QUANTITATIVE IN SITU HYBRIDIZATION STUDY. J.N. Simpson* and J.F. McGinty. Department of Anatomy and Cell Biology, East Carolina University, Greenville, NC 27858-4344. Activation of the adenylate cyclase-CAMP second messenger system leads to an increase in preproenkephalin (PPE) and preprodynorphin (PD) gene expression. Because opioid receptors are most likely involved in opioid drug actions, preopiod gene expression may be negatively influenced by opioid receptor agonists. We previously demonstrated an increase in striatal PPE and PD mRNA levels as well as an increase in striatal phospho-CREB and FOS immunoreactivity following intracerebroventricular (ICV) administration of forskolin (Simpson and McGinty, Neuroreport, June, 1994). In a preliminary experiment, medial striatal PPE and PD mRNA levels were measured in rats which received either unilateral ICV injection of soluble forskolin (FSK, 10 mM/10 ml dH2O) 1 h following systemic injection of morphine (MOR, 25 mg/kg, n = 4) or unilateral ICV FSK only (n = 4). In the MOR-FSK group, a booster injection of MOR (10 mg/kg) was given 2 h after ICV FSK. The mRNA hybridization signal in the striatum ipsilateral to ICV FSK was compared with hybridization signal in the contralateral striatum. FSK induction of PPE, but not PD, mRNA was significantly attenuated by MOR. In a separate experiment, levels of striatal pro- and zilb268 mRNA were examined 4 h following ICV FSK (10 mM/10 ml saline, n = 5) or ICV saline (10 ml, n = 5). FSK significantly induced PPE, PD, and zilb268 mRNA in the medial striatum. The finding that striatal zilb268 mRNA is induced by FSK supports previous reports that the promoter region of the zilb268 gene contains a CRE-like element. Results of a current study confirming morphine effects on forskolin-induced pro-opioid mRNA in the rat striatum as well as morphine effects on forskolin-induced zilb268 mRNA will be presented. Supported by DA05740 (JNS) and DA03892 (JFM).

556.16 MOLECULAR BASIS OF DOPAMINE/GLUTAMATE INTERACTIONS IN THE RAT STRIATUM. C. Konrad*, X.-M. Li*, R.L. Cole* and S.E. Hyman*, Laboratory of Molecular and Developmental Neurosciences, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129 and Department of Psychiatry, University of Saskatchewan, Saskatoon, SK, Canada.

Abnormalities in dopamine-glutamate transmission have been implicated in the pathogenesis of neuropsychiatric disorders including Parkinson's disease and schizophrenia. The goal of our studies is to elucidate the role of glutamate in drug-mediated gene regulation in striatum. Knowledge of dopamine-glutamate interactions may provide novel targets of drug therapy. We have previously demonstrated regulation of AP-1 binding proteins in rat striatum by dopamine agonists and antagonists. In addition, we and others have observed that D1 and D2 dopamine receptor-mediated c-fos expression is inhibited by pretreatment with the NKMDA receptor antagonist MK-801. Conversely, in primary striatal cultures, low levels of glutamate that are insufficient to induce c-fos expression significantly enhance dopamine mediated c-fos expression and AP-1 binding activity. This effect appears to be mediated by Ca2+ binding proteins, while glutamate has no effect on cAMP levels. In addition, the Ca2+ ionophore A23187 was able to mimic the effects of glutamate. These data suggest that interactions between Ca2+ and the cAMP pathway may be critical in the regulation of striatal gene expression in response to dopaminergic drugs.
557.1

BEHAVIORAL AND BIOCHEMICAL PROPERTIES OF GABA AND BENZODIAZEPINE RECEPTORS IN CBA AND C57 MICE.

C57B1/6 male mice show "normal" habituation in a locomotor activity test situation, whereas CBA/Ca background animals, CBA mice also display increased latency in a passive avoidance situation and an increased sensitivity to the convulsive actions of the GABAergic antagonist picrotoxin. Receptor binding studies revealed no differences in benzodiazepine (BDZ) binding properties or in the ability of various BDZ receptor ligands to inhibit BDZ binding.

Neither was there any differences in GABA- or pentobarbital-produced enhancement of BDZ binding. However, the binding of 3HBDP (2 nM) was about 30% higher in forebrain microsas of CBA mice as compared to C57, which was not strain difference, however in midbrain or DMCM-produced modulation of 3HBDP binding. Further experiments revealed no differences in GABA-stimulated 3Hcholine in CBA mice. However, midbrain-admained enhanced of 3Hcholine influx was significantly higher in forebrain microsas of C57 mice as compared to CBA mice. In contrast, the DMCM-produced a more marked reduction of GABA stimulated 3Hcholine in forebrain microsas of CBA as compared to C57. Our current data indicate that genetic differences in the GABAergic receptor system may explain at least some of the previously observed behavioral differences in the behavioral actions of BDZ receptor ligands in CBA and C57 animals.

557.2

FELBAMATE, A NOVEL ANTIPELLEPTIC AGENT, DOES NOT AFFECT COGNITION IN RODENTS (ID Smith*, ME Ozeki and VL Coffin). Scheinberg-Plough Research Institute, Kenilworth NJ 07033.

Felbamate (5-phenyl-1,3-propanedione dicarboxamide; Felbatol, Talacon) is a novel antiepileptic agent recently approved by the FDA for treatment of epilepsy in the U.S. While the mechanism of action of felbamate is not yet fully elucidated, recent evidence has accumulated that felbamate may act at the strychnine-sensitive glycine-binding site on the NMDA receptor complex. Since the NMDA receptor has been implicated in cognitive processes (i.e., LTP), the current study was designed to investigate the potential effects of felbamate on cognitive function.

Doses of felbamate up to 1000mg/kg did not produce deleterious effects in either mice or rats in passive avoidance tasks at the responding (PAR). In contrast, the noncompetitive NMDA antagonist MK-801 (dizocilpine) produced performance deficits at doses from 0.1 to 1.0mg/kg in both rats and mice. Both drugs prevented NMDA-induced convulsions (ED50=20.3 and 0.62, respectively). The therapeutic index (ratio of effective to anticonvulsant doses in ED50) for dizocilpine was less than one-fold, while felbamate had a greater than 50-fold separation. Taken together, these results indicate that felbamate does not produce cognitive deficits at doses more than 50 times the dose needed to block seizure activity in rodents.

Animal | Therapeutic Ratio
---|---
rat (24h) | 0.119 | >50
mouse (24h) | 0.078 | >50
mouse (1h) | 0.354 | >50

*Dose producing PAR deficit/Dose to block NMDA-induced seizure

557.3

INHERITABLE INDIVIDUAL-SPECIFIC BEHAVIORAL FEATURES OF RATS AND EFFECTS OF PROPOFOL.
R. Drinker 1, B. Elfenbrock 2 and A.R. Cools 3.

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Inheritable differences in the physiological state of the brain that direct the display of behavior include those in the GABA transmission. This transmission is essential to the effects of many anesthetic, including propofol. The present study uses the 14th generation pharmacologically selected APO-UNSUS and APO-SUS rats to evaluate whether the neurobiological profile of individual-specific features affects the response to propofol. We assessed the duration of abolishment of propofol-induced movements (APM) and the incidence of propofol-evoked behavior after three different doses i.v., each injected at a one week interval. One week later, the rats were anesthetized with isoflurane and the effects of propofol on withdrawal reflexes, heart rate and blood pressures were measured. The induction of APM and magnitude of reflex inhibition representing the anesthetic equivalent of effect were not different for the two types of rats. APO-UNSUS rats showed a greater heart rate variability and their systolic blood pressures were lower than APO-SUS rats. Propofol reduced heart rate variables stronger in APO-UNSUS rats, whereas blood pressure variables were reduced to a greater extent in APO-SUS rats. Cardiovascular responses serve to indicate the sensitivity of an individual to an anesthetic in clinical conditions. Intravenous injection of propofol produced differences occurred at the same magnitude of anesthetic responses. Drug induced differences were observed in the two types of rats. APO-SUS rats showed a higher incidence of involuntary muscular contractions after i.v. injection of propofol, whereas the incidence of grooming behavior was lower. The difference in these adverse reactions may relate to the mirror image in functionality of GABA transmission after the nico-oligos pathways for the two types of rats. Translated to the human condition, personality may affect the severity of side-effects of propofol.
MODULATION BY INOSITOL OF CHOLINERGIC AND SEROTONERGIC-INDUCED SEIZURES IN LITHIUM TREATED RATS. M.B. Williams* and R.S. Jope. Dept. of Psychiatry and Behavioral Neurology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Lithium's inhibitory effect on inositol monophosphatase of the PI pathway was suggested to be involved in the mechanism of action of lithium treatment for bipolar affective disorder (Berridge et al., 1982). Inositol depletion occurs following toxic but not therapeutic doses of lithium. However, supplemental inositol attenuated seizures induced by acute lithium plus pilocarpine (PILO) (Tricklebank et al., 1991; Koffman et al., 1993). We used EEG measurements of seizures produced by PILO given to lithium-pretreated rats (acute or chronic) and determined the effect of myo- and epi-inositol on seizure latency. Myo-inositol (0.55 mmole, i.c.v) dose-dependently increased latency to seizures induced by acute lithium (3 mmole/kg, i.p) plus PILO (30 mg/kg, i.p). Epi-inositol, which is not utilized for PI synthesis, blocked seizures in 50% of tested rats. In chronic lithium treated rats, inositol did not block seizures produced by PILO. Similarly to its effect on muscarinic agonists, lithium increased the response to R(-)-DOI, a selective 5HT2 (PI-linked) agonist (Williams and Jope, 1994). While DOI (5 mg/kg, i.p.) alone was not convulsant, lithium plus DOI evoked seizures. Myo-inositol (i.c.v) blocked seizures in some rats treated with lithium and DOI.


The ability of many antidepressant treatments to increase synaptic concentrations of serotonin is believed to be the mechanism by which they exert their clinically beneficial effects. It is uncertain, however, via which receptors the increased concentrations of serotonin subsequently act. Several studies have shown that agonists at 5-HT1B receptors such as RU 24969 demonstrate antidepressant-like actions in the mouse tail suspension test. We set out to determine whether such treatments reduce immobility in the mouse tail suspension test and to compare the effects of two recently described compounds; WAY 100135 and GR 127935, selective agonists at 5-HT1A and 5-HT1B receptors respectively, on the effects of this and other antidepressant treatments. All drugs were administered s.c. RU 24969 significantly decreased immobility at doses of 1 or 3 mg/kg. Neither WAY 100135 (10 mg/kg) had any effect in this test up to doses of 10 mg/kg. WAY 100135 (10 mg/kg) had no effect on the decrease in immobility induced by paroxetine (1 mg/kg) or RU 24969 (3 mg/kg), thus we conclude that the effect of RU 24969 on the 5-HT1B effects of the 5-HT1B agonist but also blocked the effects of paroxetine (1 mg/kg) and imipramine (10 mg/kg) in the TST. In conclusion, these results indicate that the 5-HT1B/D receptor may be involved in the mediation of the behavioural action of antidepressant agents.
557.11
EFFECTS OF CORTICOSTERONE AND DEXAMETHASONE ON SCHEDULE-INDUCED POLYPсидIA IN ADRENAL CohEIZED RATS. F. Crippa1, H. van Oers1, E. R. De Kloet1 and S. Levine1.

Removal of circulating glucocorticoids by adrenalectomy prevents the normal acquisition of schedule-induced polydipsia (SIP), while corticosterone (CORT) administration reinstates this behavior in adrenalectomized (ADX) rats. These studies investigated which glucocorticoid (GC) receptor is responsible for mediating CORT effects on SIP. In Experiment I the effects of dexamethasone (DEX) and CORT on the acquisition of SIP were studied. DEX and CORT pellets (respectively, 15 mg and 200 mg) were implanted subcutaneously in ADX rats. CORT but not DEX replacement was able to reinstate SIP in ADX rats. Because DEX binds almost exclusively to glucocorticoid receptors (GRs), while CORT binds to both GRs and mineralocorticoid receptors (MRs), results from Experiment I indicate that GRs alone are not sufficient for SIP acquisition. In Experiment II CORT pellets of different concentrations (1, 10, 30, 200 mg) were implanted in ADX rats in order to distinguish whether MRs alone, or a combination of GRs and MRs is required for SIP reinstatement. Results from Experiment II showed that the 1 and 10 mg CORT pellets were not able to reinstate SIP in adrenalectomized rats, while animals implanted with 50 or 200 mg pellets did show the behavior. These results indicate that occupancy of both MRs and GRs is required for SIP acquisition.

557.12
3α-ANDROSTANE30 DIOL INHIBITION OF SEXUAL RECEPTIVITY AND NOCICEPTION: EVIDENCE FOR A GABAERGIC MECHANISM. C.A. Frye1, 2, K. V. Kueken and M. S. Erkens1.
1 Department of Biology, Boston University; 2 Cumming School of Medicine, Boston, MA 02215

Many steroids can act via intracellular receptors and at the GABA receptor complex (GBR). Progesterone (P) or its metabolites may initiate sexual receptivity and 3α-reduced androgens, particularly 3α-Androstan-30 one at the GBR, may produce female-like effects in some species. To investigate this hypothesis, ovariectomized rats (N=50) received 3α-Diol (0.6, 3.0, 6.0 and 7.5 mg/kg) via vehicle (10% (v/v) ethanol/proplylene glycol) at 1000h (hrs), and 2.5 SC injections of estradiol (E2; 1 μg/g; pil in 1% ethanol) at 1300h (hrs) and 1500h (hrs) daily for 2 days. P (0.5, 1.0, 2.0 and 4.0 mg/kg) or saline was given at 1400h after 2 days of 3α-Diol and E2. Behavioral testing was carried out at 1830h, 48-49 hours after the first injection of E2 at 4 hour after injection of P. 3α-Diol (3.0 and 6.0 mg/kg) significantly attenuated P (2.0 and 4.0 mg/kg) facilitation of sexual behavior (Lordosis Quotient, Lordosis Rating, Proceptivity). 3α-Diol and E2 both produced analgesia assessed via the radiant heat tail-flick method. 3α-Diol at 3.0 mg/kg inhibited P-induced, uniformly produced maximum analgesia, and dramatically increased GABA-stimulated chloride flow into cortical synaptosomes. As 3α-Diol binds poorly to its intracellular receptors, these data suggest that 3α-Diol may act via the GBR to inhibit sexual receptivity and promote analgesia.

Supported by Lilly Pharmacology Award, Luce Professorship and HD21802 to MSE and MH1074 to CAF.

557.13
THE CCK RECEPTOR ANTAGONIST CV-67736 INHIBITS ETHANOL INDUCTION OF IMMUNOCALL OF THE GLOUCOCORTICOID RELATED MECHANISM IN THE PARA-SOMNOCENTIC IN RATS. M. Hulset1, S. Bernardo1, J. M. Puntervold1, Dept. of Pharmacology, Sch. of Pharmacy, Univ. Copenhagen, Copenhagen, Denmark.

Rescue of ethanol (EtOH) withdrawal dependence by the CCK antagonist CV-67736, was assessed in the Sprague-Dawley rat model of withdrawal syndrome. EtOH withdrawal was induced by maintaining the rats on a liquid diet containing EtOH (8% v/v) for 21 days, followed by withdrawal for 5 days. Ethanol withdrawal produced a dose-dependent increase of the c-fos mRNA level in the hypothalamus, striatum, hippocampus and amygdala, but not in the cerebellum or cortex. Treatment with CV-67736 (1 mg/kg) attenuated the increase in c-fos mRNA levels in all regions except the amygdala. These results suggest that EtOH withdrawal produces a CCK-dependent increase in c-fos mRNA levels in several brain regions.

557.14
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The sulfated neurosteroid Pregnenolone Sulfate (PS) has been shown to have a variety of behavioral effects. Recently evidence has been presented that PS affects cognitive processes. The present study examined the effects of PS on spatial learning and memory in adult male rats. PS (0.5 and 1.0 mg/kg) and vehicle treated rats were tested in a Morris water maze task, where they had to learn the position of a hidden platform. PS treated rats were found to learn the task significantly faster than the saline vehicle controls, although the vehicle group had significantly better retention. The effects of PS on locomotor activity and swimming behavior were also examined. In a multivariate analysis of locomotor behavior, PS was found to cause a dose-dependent increase in activity. There were, however, no significant effects on swimming behavior. These results suggest that PS may affect spatial learning and locomotor activity. Supported by an NSERC to KPO and MK.

557.15
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The nitric oxide synthase inhibitor N-(G)-monomethyl-L-arginine (NMMA; 25, 50, and 100 μg) was microinjected into the medial preoptic area (MPOA) of male rats. In a test of ex copula sexual reflexion, restrained saline treated rats exhibited a dose-dependent increase in seminal emissions which was significant at the 400 μg dose. This effect was not mimicked by the inactive isomer N-(G)-monomethyl-D-arginine. In a subsequent copulation test, animals in the NMMA (400 μg) condition were significantly less likely to successfully initiate copulation than were animals in the vehicle condition. The results suggest that nitric oxide in the MPOA contributes to the regulation of male sexual behavior.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
558.1 ASSESSMENT OF DRUG-INDUCED REBOUND USING A THREE-LEVER DRUG-DISCRIMINATION TASK. W. E. Cast, R. J. Barrett, E. M. Huffman, and J. R. Stadler. Department of Psychology and V. A. Medical Center, Vanderbilt University, Nashville, TN 37240. Our previous research, using a two-lever drug-drug discrimination task, has shown that time-dependent, bidirectional changes in choice responding occur following administration of drug, EEDQ, and psychomotor stimulants. Because one limitation of the two-lever procedure is that it is not possible to identify cue states that differ from those associated with the training drugs, in the present study animals were trained on a three-lever task to discriminate among two drugs and vehicle.
Rats were trained over a 10-month period using a VI schedule of food reinforcement to discriminate between 0.5 mg/kg amphetamine, distilled water, and 0.03 mg/kg haloperidol. The dose-response functions for responding on each lever indicate that animals were responding on the basis of a continuum of dopaminergic activity. Subsequent to a large (10 mg/kg) dose of amphetamine, predominant responding shifted from the amphetamine lever at 9 hr, and then to the haloperidol lever at 20 and 24 hrs.


The behavioral effects of caffeine were studied alone and in combination with methamphetamine (MA) in squirrel monkeys trained to discriminate 0.30 mg/kg MA from saline using conventional drug discrimination procedures. In initial experiments, caffeine (3.56 mg/kg) substituted for MA to varying extents in four of seven monkeys, resulting in 67-100% MA-lever responding. However, the MA-like discriminative stimulus effects of caffeine diminished over successive determinations and could not be reinstated regardless of dose. Pretreatment with caffeine (10 and 4 mg/kg) did not systematically alter the discriminative stimulus effects of MA; on the other hand, pretreatment with a normally ineffective dose of MA did partially restore caffeine's MA-like effects (50-90% MA-lever responding). These findings suggest that, although caffeine and MA generally have dissimilar discriminative stimulus effects, their behavioral actions may overlap to a limited extent. Supported by DA 03774, RR00168, DA 00499 and MH 07658.

558.5 EFFECT OF FOURPHET, AN IRREVERSIBLE INHIBITOR OF STIMULANT BINDING, ON OCAMINE-INDUCED BEHAVIORS. N. Scholl* R. B. Gupta, and E. Asch, Div. Basic Med. Sciences, Medicine, UC-SF, Sch. Med., San Francisco, CA 94143. We have previously shown that 20 mg/kg (I.v.) Fourphet HCl (4-isoethyl-4-(1-methylcyclohexyl)pyridinidide; FPH), an irreversible inhibitor of [benhyl]methylphenidate binding to the stimulant recognition site on the dopamine transporter, decreases the total, ambulatory, and non-ambulatory movements caused by a single dose (15 mg/kg, s. p.) of cocaine/HCl (COC) 24 hrs after treatment with FPH. The present work examined the effect of the same dose of FPH on the behavioral responses to a high dose of COC (40 mg/kg, s. p.) of cocaine/HCl (COC) 24 hrs after treatment with FPH. The present work examined the effect of the same dose of FPH on the behavioral responses to a high dose of COC (40 mg/kg, s. p.) of cocaine/HCl (COC) 24 hrs after treatment with FPH. After overnight acclimatization in individual activity monitor cages, male Sprague-Dawley rats were injected with FPH or vehicle (VEN; 20% EtOH/saline), then challenged 24 hrs later with 40 mg/kg (s. p.) COC. Activity counts recorded after COC injection were analysed by 2-way (time x treatment) ANOVA with repeated measures on the first factor. Significant time x treatment interactions were obtained for the total (p<0.05) and ambulatory (p<0.05) activity counts recorded at these consecutive 25 min intervals, starting at 10 min after COC administration. Analysis by two-way ANOVA showed that FPH-treated rats were significantly less active than VEN controls during the first measurement interval. Although FPH had no effect on nonambulatory activity (often used as a measure of stereotypy), differences in particular stereotypic behaviors were found: FPH-treated rats exhibited more thigmotaxis (p<0.03) and less rearing (p<0.04) after COC than controls. The data show that FPH has a significant effect on behaviors caused by a high dose of COC. (Supported by NIDA #DA00305 and NINDS #NS28258).

558.6 MODULATION OF DOPAMINE RECEPTOR AGONIST INDUCED ROTATIONAL BEHAVIOUR BY PLG AND ITS NOVEL ANALOGUE PAPOA. D. When, R.K. Mishra* and R.L. Johnson. McMaster Univ., Hamilton, Ont. & Dept. of Medicinal Chemistry, Univ. of Minnesota, Minneapolis, Mn.
The interaction of L-prolyl-L-leucylglycinamide (PLG) and its novel synthetic analogue 3-(R)-[N-(L-prolyl)aminocarbonyl]-2-oxo-1-phenylalanine (PAPOA) with central dopaminergic receptors was investigated in 6-hydroxydopamine lesioned rats. PLG maximally potentiated amphetamine-induced rotations at a dose of 1.0 mg/kg (intraperitoneal injection, ip) and 100.0 ng (intrastriatal injection, i.s.). PAPOA was 100% more potent than PLG, inducing maximal potentiation at dosages of 10.0, 5.0, and 1.0 ng (i.s.) Co-administered intrastriatal injections of PLG and PAPOA (100.0 ng and 1.0 ng respectively) enhanced the agonist-induced rotational responses by 25% and 50%, respectively. These injections of PLG and PAPOA (1.0 mg/kg and 1.0 mg/kg respectively) similarly increased the rotational response by 50%. PAPOA also potentiated both SKF 38390 and quinpirole induced rotational responses by 50% and 100%, respectively. PAPOA maximally mediated its effects via D1 dopamine receptors as PAPOA produced an 85% increase in quinpirole (D1 agonist) induced rotations. In contrast, PAPOA yielded a modest 14% increase in SKF 38390 (D2 agonist) induced rotations. Haloperidol blocked these peptide potentiated dopamine actions and those induced by quinpirole regardless of the route of administration (i.p. or i.s.). D1 25330 blocked both D1 and D2 actions potentiated by PAPOA. Thus PAPOA, with greater potency than PLG to modulate the effects of dopamine agonists, is of therapeutic importance in the treatment of Parkinson's disease and the dyskinesic syndromes. (Supported by NIH Grant NS 20563).
558.7 DIZOCYLINE (MK-801)-INDUCED BEHAVIOR IN RATS IS INHIBITED BY NEUROLEPTIC DRUGS AND POTENTIATED BY ACVINIC. P. Andiné*, R. Axelson and M. Sandberg. Departments of Clinical Neuroscience, Section of Psychiatry, Sahlgrenska University Hospital, and Anxiety and Cell Biology, Institute of Neurobiology, University of Göteborg, S-413 90 Göteborg, Sweden.

N-Methyl-D-aspartate (NMDA) receptor antagonists are psychotomimetics offering the most schizophrenia-like drug-induced syndrome in humans. The NMDA antagonist dizocilpine (MK-801) causes a typical behavior in rats which is used as an experimental model for psychosis. We used adult Sprague-Dawley rats and observed them from 15-75 min after intraperitoneal administration of MK-801 (0.05-1.0 mg/kg). They were scored for locomotion, stereotyped sniffing and ataxia every 5 min throughout the experiment. MK-801 caused altered locomotion and stereotyped sniffing in lower doses and also ataxia in higher doses. Female rats were much more susceptible to MK-801 than males. Neuroleptic drugs, administered in a single-dose 30 min before MK-801, offered a potent reduction of MK-801 behavior, with a total inhibition at doses (haloperidol 1.0 mg/kg, perphenazine 2.0 mg/kg, chlorpromazine 10 mg/kg, remoxipride 20 micromoles/kg) that corresponded to their anti-psychotic potency in patients. An enhancement of MK-801 behavior was found in male rats pretreated with acvinic (100 mg/kg), an inhibitor of the putative amino acid reuptake enzyme gamma-glutamyl transferase. In vitro, acvinic did not influence extracellular (ec) glutamate, however, it increased ec glutathione. Glutathione may cause NMDA receptor blockade via redox reactions and thereby an enhancement of MK-801 behavior. In conclusion, MK-801-induced behavior in rats is aggravated by gamma-glutamyl transferase inhibition and abolished by neuroleptics.

558.8 THE EFFECTS OF DOPAMINERGIC AGONISTS ON DELAYED MATCHING-TO-SAMPLE (DMS) PERFORMANCE UNDER THERMAL NEUTRAL AND COLD STRESS CONDITIONS. D. Shurtleff*, J. R. Thomas, S. T. Albers, and J. Schott Naval Medical Research Institute, Bethesda, MD 20889-5607.

Dopaminergic drugs have been shown to be important in the modulation of working memory. This study examined the role of the D1/D2 agonist quinpirole (0.025-0.1 mg/kg) and the D2 agonist quinpirole (0.05 mg/kg) on working memory in rats using a DMS procedure. Each DMS trial required rats to respond to one of two cued levers, which initiated a variable delay of 1-16 sec. Following the delay, rats were reinforced for correctly responding to the lever. Under thermal neutral conditions (22°C) following saline, rats' matching accuracy showed a characteristic decline as the delay interval increased. Consistent with previous research and relative to 22°C, matching at 2°C (cold) was reduced. Both drugs increased matching accuracy at higher doses. Quinpirole did not prevent the cold-induced decline in matching. SKF 38393 did not reduce matching accuracy at 2°C and did not affect matching accuracy at 2°C. Drug and cold effect results include the analysis of changes in slope (retention) and y-intercept (stimulus encoding) of the matching accuracy gradient. These data suggest that D1/D2 receptors are more important in modulating working memory than the D3 receptor, and these receptors may not be directly involved in cold-induced working memory deficits.


The contribution and optimum mode of stimulation of dopamine D2 receptors in the treatment of Parkinson's disease (PD) remain poorly defined. To define stimulation mode in one experiment, the long-acting, selective D2 agonist A77776, N,N,N,N-tetrahydro-3'-[[(4-(dihydrol-5,6-dihydroxy-1H-benzazepin-2-yloxy)[1,4]diazine hydrochloride] (Kebabian et al., JPB 1992; 229; 203) was administered daily for 7 days at doses ranging from 0.05 to 10 mg/kg, s.c., in MPTP-exposed, parkinsonian cynomolgous monkeys with levodopa-induced dyskinesia (UD). Following the first dose (0.5 mg/kg), all monkeys quickly showed a definite antiparkinsonian response for at least 8-12 hrs with increased ambulation persisting overnight. Only mild dyskinesia and stereotypies were seen. A second dose the next day was not as effective, and the level of motor activity progressively declined in 3 animals but remained above baseline values during the last 5 days of treatment. In spite of the active administration of high doses, in another parallel paradigm, the short-acting D2 agonist SKF 82958 (6-bromo-7-hydroxy-3,4-alkyl-1-phenyl-2,3,4,5-ethylhydro-1H-benzazepine hydrobromide) was administered to drug-naïve, MPTP-exposed, parkinsonian cynomolgous monkeys at 4 doses ranging from 0.025-0.1 mg/kg s.c. Three days for 4 weeks of intermittent administration were given in all animals for 30-50 minutes following each dose. However, after several days of treatment and especially in the last 2 weeks of the protocol, the drug response significantly shortened (20-30 min) in all animals and choreomimic dyskinesia developed in 2 white park response was maintained. These data clearly shows that selective D2 receptors stimulation can effectively relieve parkinsonian features and lead to dyskinesia similar to that seen in PD over long periods of treatment. D2 receptor occupancy should be avoided. The mechanisms for such pharmacological behavior and ways to prevent it must be explored further. [Supported by FRSG and MRC (Canada)].

558.10 BEHAVIORAL AND BIOCHEMICAL STUDIES ON THE INTERACTION OF THE NMDA ANTAGONIST MK-801 AND THE D2 ANTAGONIST HALOPERIDOL. H. Dai*, R.J. Carey, VA Medical Center, Research Service 151, Syracuse, NY 13210.

Thirty two male rats were assigned to four groups and were given saline, MK-801 (0.3 mg/kg), haloperidol (0.5 mg/kg) and MK-801/haloperidol combined treatments. The behavioral data of locomotor activity showed that the NMDA antagonist MK-801 induced hyperactivity, haloperidol induced hypoactivity but that the activity of the combined MK-801/haloperidol group was identical to the saline animals in both total locomotion distance and the within session habituation pattern. On the other hand, the Ex vivo biochemical data revealed no significant effect by MK-801 on dopamine metabolism, nor did MK-801 modify haloperidol effects on dopamine metabolites turnover ratios. None of the drug treatments had an effect on brain serotonin metabolism. Haloperidol, however, significantly increased serum 5-HT level. No drug treatment affected plasma catecholamine or plasma corticosterone levels. Taken together all these results supports the hypothesis that the effect of MK-801 on behavior is independent of biochemical changes in dopaminergic transmission.


The purpose of this study was to determine whether amphetamine-treated rats can learn to suppress stereotypy head movements. Rats implanted with cannulas were reinforced with intravenous infusions of milk for holding their heads stationary within a narrow area defined by intersecting photobeam mounted in the horizontal and vertical planes. Concurrent interference of these photobeams activated an infusion pump, which delivered milk at the rate of 2 cc/min for the duration of the interruption. Each rat was given chronic infusions of amphetamine (2 mg/kg) learned to hold their heads within the intersecting photo beams. The amount of milk ingested as a result of these photobeam over trials at a rate that was comparable to that of bottle-fed rats. Analysis of the temporal distribution of photobeam interruptions showed a highly frequency pattern that differed markedly from the sustained pattern found in saline controls. These results support the view that the locomotor and stereotypic effects of amphetamine involves an instrumentally learned behavioral adaptation.

(Supported by NIDA grant DA 04592)

The effects of a reference neuroleptic, haloperidol, were compared with those of three atypical antipsychotic agents (clozapine, DuP 734 and risperidone) and a S-HT₂ antagonist, ritanserin, in a number of procedures. Drugs were first examined in mice over a wide dose range using a primary observation method in order to target the doses for other studies. Haloperidol (0.125-2 mg/kg) was distinctly active in all procedures except for marked catalepsy. Risperidone was more potent than the other compounds in many of the procedures, often having activity at doses well below 1 mg/kg. Neither clozapine (1.0-16 mg/kg) nor DuP 734 (0.5-8 mg/kg) induced catalepsy in rats yet both drugs appeared to antagonize apomorphine-induced hyperactivity at doses less effective against apomorphine- or apomorphine-induced stereotypes in mice. Clozapine and DuP 734 along with ritanserin, also clearly blocked mescaline-induced scratching. Ritanserin (2-16 mg/kg), in general, did not have a behavioral profile like those of the other four drugs. All compounds also differed with respect to their actions as antagonists of PCP-induced stereotypes and SKF 10047-induced hyperactivity in rats. In view of the clinical findings currently available with these drugs, it appears highly useful to employ a series of methods in order to identify novel antipsychotics that differ from haloperidol. Absence of cataleptogenic properties of apomorphine-induced hyperactivity would appear to be useful criteria.

Effect of NO synthase inhibition on behavioral changes induced by a single administration of methamphetamine. Tomohiro Abe*^tai, Teppei Ohmori and Touruaka Koyama.

Department of Psychiatry and Neurology, Hokkaido University School of Medicine, Sapporo, Japan

The present study examined the effects of nitric oxide synthesis inhibition on behavioral changes induced by a single administration of methamphetamine (MA). Rats received one subcutaneous injection of MA (3.22 mg free base/kg). Nitric oxide synthase inhibitor, Na-nitro-L-arginine methyl ester, LNAME (10,30 and 60 mg/kg) were administered intraperitoneally 1 hr prior to injection of MA or saline. MA increased locomotion-stereotypy rating scores and motor activity counts measured by an infrared sensor. LNAME (30 and 60 mg/kg) administered 1 hr prior to MA significantly decreased the level of locomotion stereotypy rating and motor activity induced by MA. The results suggest that NO synthesis is involved in the full expression of behavioral effects of MA.

The noncompetitive NMDA receptor antagonist (+)-disocilpine fails to prevent sensitization to apomorphine-induced lever-rotational behavior in rats with nigrostriatal lesions. S Gancher* and A Mayer, Dept of Neurology, Oregon Health Sciences University, Portland, Oregon, 97201.

Sensitization to CNS stimulants is well described and is reportedly prevented by the noncompetitive NMDA recep- tor antagonist (+)-disocilpine (MK-801). We sought to deter- mine if MK-801 pretreatment prevents sensitization to apomorphine (AP0)-induced rotational behavior in rats with unilateral nigrostriatal lesions. Six rats were lesioned with 6-OHDA, and beginning 2 weeks later, received 8 daily doses of MK-801 (0.1 mg/kg, ip) followed 30 min later by AP0 (0.1 mg/kg, sc). Three and 5 days later, single doses of the D1 agonist SKF38393 (2 mg/kg) AP0 (0.1 mg/kg, day 5) were administered with or without MK-801 pretreatment. Despite MK-801 pretreatment, the rotational behavior increased, with a peak over the protocol (for doses 1, 4, and 8, total and peak/5 min rotations were 104 and 24, 407 and 92, and 576 and 85, respectively, p=0.05). The dose of SKF induced 1624 rotations. MK-801 enhanced AP0 rotations; in comparison with AP0 alone, AP0 alone induced 449 rotations (p=0.05). In a separate experiment, 3 rats received MK-801 + AP0 on postlesion day 14, and 3 days later, received 2 mg/kg SKF 38393; as reported by Morelli et al, SKF 38393 failed to induce rotational behavior. These data suggest that in contrast to sensitization in unlesioned animals, lesioned animals sensitive to AP0 despite NMDA channel blockade.

QUINOLINIC ACID-INDUCED LESION OF THE SUBSTANTIA NIGRA PARS RETICULATA: BEHAVIOURAL AND NEUROCHEMICAL EFFECTS. B. zdazov*, M. Bubser and W.J. Schmidt. Department of Neuropsychology, University of Tübingen, D-72074 Tübingen, Germany.

As one of the major output structures of the basal ganglia (BG) the substantia nigra pars reticulata (SNr) is part of the motor loop through the BG and thus is involved in the performance of motor behaviour. The SNr receives information from the striatum and sends inhibitory GABAergic projections to the thalamus and the deeper layers of the superior colliculus. The present study investigated the role of the SNr in mediating motor behaviour in male Sprague Dawley rats. The SNr was bilaterally lesioned with the excitotoxin quinolinic acid (2 x 30 nmol/5 μl side). Locomotor activity and exploration were tested in an open field with holeboard and sniffing stereotypy was tested in an experimental chamber (Schmidt, W.J., 1986, Psychopharmacology 90: 123-130). Both locomotor- and sniffing activity were increased after SNr lesions, whereas exploration was not changed. The histological verification of the SNr lesions demonstrated that in most cases a part of the substantia nigra pars compacta was also lesioned. The levels of dopamine (DA) and its metabolites (DOPAC, HVA) in the anterior and posterior striatum and in the nucleus accumbens were analysed post mortem with HPLC with electrochemical detection. DA and DOPAC were reduced in all three structures, whereas HVA was only reduced in the anterior striatum. The metabolism of dopamine (measured as DOPAC/DHA and HVA/DHA) was increased in the anterior and posterior striatum. These increases correlated significantly with the increases in locomotor- and sniffing activity. In conclusion, bilateral lesions of the SNr and thus reduced activity of this structure results in an increase of motor activity. This increase may be explained by the deficient inhibitory effects of the SNr on the thalamus and the superior colliculus, but also by the increased dopamine metabolism in the striatum. Supported by EMPT 01KL90080 and SFB 307/4A.

POTENTIATION OF THE D-HYDROXYDOPHINE DISCRIMINATIVE STIMULUS BY PHOSPHODIESTERASE INHIBITION AND THE EFFECT OF RECEPTOR RESERVE ON D₂ AGONIST SUBSTITUTION, Q.D. Walker¹, D.M. Black¹, D.A. Eckerman¹, D.E. Nichols¹ and R.B. Malinak¹. Curriculum in Toxicol., Univ. of N. Carolina, Chapel Hill, NC 27599; and Dept. Med., Chem. Purdue Univ², West Lafayette, IN 47907.

In rats, administration of the full D₂ agonist dihydroxypoline (DHP, 2 mg/kg) induces robust discriminative stimulus (DS) effects that are mediated selectively by D₂ receptor stimulation. We investigated whether the DHP cue was also mediated by increased intracellular concentrations of cAMP. The phosphodiesterase inhibitor rolipram (0.75, 15, or 30 μg/kg) was administered either alone or in combination with doses of DHP (0.25 or 0.5 mg/kg or SKF38393 (1.0 or 2.0 mg/kg) that induce little drug appropriate responding. Rolipram (15 μg/kg) and DHP (0.25 mg/kg) induced 0 and 7% DHP lever responding, respectively, when administered alone, but 50% when coadministered. Rolipram also synergistically increased SKF38393 substitution. Coadministration of rolipram (50 μg/kg) and SKF38393 (2.0 mg/kg) induced 67% drug lever selection, although the compounds induced only 15 and 29% drug lever selection, respectively, when administered alone. The second series of experiments tested the hypothesis that D₂ receptor reserve explains the complete substitution of the partial agonist SKF38393 for the DHP cue. EEDQ (10 mg/kg) was administered with a receptor protocytic cocktail (pirazin, ketanserin, remisedrine) to decrease D₂ receptor number by about 50%. Forty-eight hr after EEDQ, rats were administered desipramine (10 mg/kg) that substituted >90% prior to EEDQ treatment. Administration of EEDQ significantly decreased the substitution of SKF38393 (97% vs. 26%) but did not affect substitution of DHP (98% vs. 74%). These results coupled with information to mediate the DS effect by D₂ and that SKF38393 substitutes for DHP by occupying a greater proportion of the DS receptor population than does DHP.
559.1


Much evidence suggests that glutamate plays a role in the spinal transmission of the reflex autonomic responses to static contraction. Previously we measured the reflex increase in arterial pressure evoked by static contraction of the hindlimbs muscles in chloralose-anesthetized cats before and after glutamatergic receptor blockade in the lumbosacral cord. The reflex pressor response in this reaction was unaffected by NMDA receptor blocker AP-5 (500 μg, i.v.), but was significantly attenuated by non-NMDA receptor blocker CNQX (10 μM, i.v.). These findings suggest that the glutamatergic reflex activation of the spinal cord was mediated by non-NMDA receptor activation, possibly through NMDA receptors activated by the glutamatergic neurotransmitter system.

559.2

SPINAL RELEASE OF SUBSTANCE P DURING MUSCLE CONTRACTION: EFFECT OF S-HT4 RECEPTOR ACTIVATION. A.C.L. Nogueira, A.F. Menin, A. Akly, L.B. Wilson. Harry S. Moss Heart Center, University of Texas Southwestern Medical Center, Dallas, TX 75235-9934.

Static muscle contraction increases mean arterial pressure (MAP) and substance P (SP) release in the dorsal horn of the spinal cord. Activation of 5-HT4 receptors in this region attenuates the reflex pressor response to contraction. This study evaluated the effect of microinjection of 8-hydroxy-2-(dipropylaminophenyl)tetralin (DPAT; 5-HT4 agonist) into the L6 dorsal horn on the release of SP during contraction using anesthetized cats. Ipsilateral to the muscle to be contracted, all ventral and dorsal roots from L6 to S1 were severed, except the L6 dorsal root. Static contraction of the triceps surae muscle was induced by alternate electrical stimulation of the distal ends of L6 and S1 ventral roots for 5 min (3X motor threshold; 30 Hz; 0.1-0.3 mA). All the discharges from a pair of probes, placed in the L6 dorsal horn region ipsilateral to the contracting muscle, were analyzed for SP-like immunoreactivity (SP-LI) using a radioimmunoassay. DPAT (10 μM, n = 8) attenuated the MAP response to contraction (control: 38 ± 11; DPAT: 10 ± 3 mmHg; P < 0.05). Likewise, the heart rate response was also blunted (control: 13 ± 3; DPAT = 7 ± 3 bpm; P < 0.05). However, the SP-LI values during contraction were unaffected by DPAT (control = 0.46 ± 0.028; DPAT = 0.501 ± 0.034 fmol/100 μL; P > 0.05). These results suggest that the modulation of the pressor response to contraction by 5-HT4 receptors in the dorsal horn is not mediated through inhibition of SP release.

559.3

SYSTEMIC 2-DEOXY-D-GlUCOSE LEADS TO EXCITATION OF ADRENA L ME DULLARY SYMPATHETIC PREGANGLIONIC NEURONS. S.F. Morrison, Dept. of Physiology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Adrenal medullary release of epinephrine and the subsequent stimulation of glycolysis and gluconeogenesis are important components of the physiological response to hypoglycemia. To determine the central pathways controlling catecholamine secretion by the adrenal medulla, the responses of the adrenal medullary sympathetic preganglionic neurons (AdSPNs) to 2-deoxy-D-glucose (2-DG) administration (200mg/kg, iv.) were studied in urethane-chloralose-anesthetized, artificially-ventilated rats. AdSPNs were identified by their antidromic responses to adrenal nerve stimulation and were localized in the rostral ventrolateral medulla (RVM). Ten minutes after 2-DG injection, the basal discharge rate of AdSPNs had increased (p<0.001) from 5.8 Hz (control) to 9.2 Hz. Subsequent bilateral intracarotid injection of musculin (25 nmol) into the RVM eliminated the 2-DG-induced increase in AdSPN discharge, although a low level of AdSPN discharge remained. The increase in AdSPN discharge following 2-DG was not different in animals with transections of the neuraxis rostral to the superior colliculus. These results suggest that activation of AdSPNs is a brainstem-mediated component of the neural response to the blockade of glucose utilization by 2-DG and that RVM neurons may be necessary for this response. Supported by NIH HL47196.

559.4

SPINAL NMDA RECEPTOR MEDIATES c-tos EXPRESSION FOLLOWING HYPOTENSION IN THE CONSCIOUS RAT. Huang, W. and West, M.J.* Department of Medicine, University of Queensland, Prince Charles Hospital, Brisbane, QLD 4022, Australia.

Recent studies show that baroreflex-induced vasocconstrictor responses following hypotension are attenuated by the administration of the NMDA receptor antagonist AP5. We have examined the effect of intrathecal AP5 on spinal cord c-tos expression induced by hypotension in conscious rats. c-tos or saline were administered intrathecally prior to a 50 minute infusion of sodium nitroprusside which lowered blood pressure by 30 mmHg. Animals were subsequently killed under general anaesthesia and the medulla and spinal cord processed for demonstration of c-tos protein using immunocytochemistry. c-tos neuronal immunoreactivity (Fos-IR) was observed in the rostral and caudal ventrolateral medullae and nucleus tractus solitarius and in the intermediolateral cell column (IML) of the spinal cord with the highest segmental concentration in segments T7-T10. At the level of the intrathecal administration of AP5, T9, there was a significant reduction in Fos-IR cells in the IML in animals given AP5 compared to those given saline (125 ± 6.9/segment (n=3) vs 235 ± 6.5 (n=2); P<0.05). There were no differences between treated and control animals in the medulla or other segments of the spinal cord. The results show that (i) following hypotension, cells in IML of segments T7-T10 show maximal expression of Fos-IR and (ii) AP5 inhibits c-tos expression. We conclude that spinal NMDA receptors in IML of spinal cord are involved in central baroreflex control of the circulation.

559.5


Anatomically localized nitric oxide (NO) synthase activity in the spinal cord intermediolateral column. The present study was designed to investigate the possibility of the spinal NO in the central regulation of blood pressure. No. The results obtained in intracisternal (i.c.) injection of NO donor, sodium nitroprusside (SNP; 1, 5 and 15 nmol) produced dose-dependent increases in BP. The vasopressor action of SNP (15 nmol) was completely blocked by pretreatment with the N-methyl-D-aspartic acid (NMDA) receptor antagonist, D,L-2-amino-5-phosphonopentanoic acid (10 μM, i.v.). This effect was also attenuated by the vasopressor effect of SNP (i.c.), 15 nmol) was completely blocked by pretreatment with the N-methyl-D-aspartic acid (NMDA) receptor antagonist, D,L-2-amino-5-phosphonopentanoic acid (10 μM, i.v.). In addition, i.v. injection of an inhibitor of NO synthase, N-nitro-L-arginine methyl ester (20, 100 and 1000 nmol) evoked dose-dependent decreases in BP. These results suggest that NO generated in spinal cord plays a role in the regulation of BP. Furthermore, the vasopressor action of the spinal NO seems to be tonically active and to be mediated via its interaction with the glutamatergic system in the spinal cord.

559.6

SECOND MESSENGER MODULATION OF MEMBRANE POTENTIAL OSCILLATIONS IN SYMPATHETIC PREGANGLIONIC NEURONS. D. Sonnenwirth, M.L.H.J. Henkes and I.P. Remaut, Neurosciences Unit, Leibniz Research Institute, Otto-von-Guericke-University, Magdeburg, Germany.

The presence of spontaneous oscillations in sympathetic preganglionic neurons (SPNs) endows these neurons with the ability for rhythmogenesis, which can be substantially enhanced by means of drugs that either block or stimulate, ions, single spike discharge or combinations of these. This activity is thought to reflect electrolytic coupling between SPNs, the oscillation representing an action potential discharged in an neighbouring neuron. Furthermore, these oscillations and rhythmic activity are subject to modulation by neurotransmitters such as serotonin and noradrenaline (Logan et al., '93). We have utilized whole-cell recording techniques in a spinal cord slice preparation to further investigate factors involved in the regulation of rhythmogenesis in SPNs, in particular the effects of elevating intracellular calcium and cAMP. The permeability of the membrane potential forming of cAMP and cAMP has a complex blockage, bicuculline, ischaemic periods or absence of cAMP (ACPD) 100 μM). The spontaneous oscillations were relatively unaffected by nifedipine (10 μM). Superfusion of cAMP (10-20 μM) induced oscillations and rhythmic activity is produced in cAMP signalling pathway of these neurons evoked a LD evoked in the presence of cAMP.

These data suggest that rhythmogenesis in SPNs is subject to regulation being induced by intracellular calcium release and inhibited by stimulation of cAMP production (Sonnenwirth et al., '93).


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559.7

MEDULLARY STIMULATION CAN EVOKE IPSPS IN SYMPATHETIC PREGANGLIONIC NEURONES IN THE BRAINSTEM-SPINAL CORD PREPARATION. Susan A. Drooght,* S.P. Morrison, R.M. Sawyer and M.P. Gilbert, Royal Free Hospital School of Medicine, Rowland Hill Street, London. NW3 JPF U.K.

In the neonatal rat brainstem-spinal cord preparation using whole cell patch recordings, we have shown that stimulation of the retrolenticular meningeal cells elicits multiphasic EPSPs in sympathetic preganglionic neurons (SPNs) mediated by both non-NMDA and NMDA receptors (Marks and Morrison, J. Physiol. 475, 159. 1994). We report here that medullary stimulation can also evoke IPSPs in SPNs. IPSPs were identified on the basis of their antidromic activation following stimulation of the ventral root and their morphology and location within the spinal cord. Stimulation of the retrolenticular meningeal (RLM) pulse (6 ms, 100-1000 μA) elicited IPSPs in 10 SPNs at holding potentials more positive than -50 mV. The mean latency to onset of these responses was 34.4 ± 4.1 ms (mean ± S.E.M.) and the amplitude at membrane potentials of -40 mV was 5.77 ± 1.0 mV. These IPSPs persisted in the presence of the excitatory amino acid receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D-2-amino-5-phosphonovaleric acid in all but one of the SPNs tested. Indeed, sometimes blockade of the EPSP was necessary to reveal an IPSP. In the presence of these antagonists, hyperpolarizing the neurons decreased the amplitude of the IPSPs until reversal occurred at membrane potentials more negative than -60 mV. On two occasions both applications of 5 μM bicuculline blocked the evoked IPSP. These results indicate that medullary stimulation can evoke IPSPs in SPNs which involve activation of GABA receptors.

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559.9


The anti-dopamine beta-hydroxylase immunotoxin (DHB) is a recently developed neurotoxic lesioning tool. In the first study, we examined the degree of sympathetic achieved in adult and neonatal rats given DHB peripherally. 30 μg of DHB was injected i.v. into anesthetized, adult, male Sprague-Dawley rats. 7 days later animals were unanesthetized and cannulated. 3 days later DHB was injected subcutaneously and 4 days later DHB was injected with a 10 day latency. Plasma was drawn before and after tyramine (300μg/kg) administration to document residual and tyramine evoked peripheral responses. DHB treated animals had significantly less basal (33%) and tyramine stimulated (20%) plasma norepinephrine levels than controls. Plasma epinephrine was unchanged. Basal blood pressure and heart rate were not significantly changed. One day old neonatal rats were injected subcutaneously with 4μg of DHB and sacrificed at 1,3,6, and 13 days afterward. Nissl staining of superior cervical ganglia from day 3 animals showed no differences compared to sham operated controls. In the neonatal study, sequential doses of DHB may be necessary to lesion remaining neurons and adrenal chromaffin cells in adults. The ability of a single dose of DHB to deplete adrenal catecholamines in neonates is an advantage over previous sympathetic pathway methods.

559.11

MEMBRANE PROPERTIES OF SYMPATHETIC GANGLION CELLS IN GENETICALLY HY珀TENSIVE OR HY珀RATIVE RATS. A.P. Gokin1,2,3 and C.J. Forehand1. Bipolom ometz Inst. of Physiol., Kiev, Ukraine, 262024 1Dept. of Anat. and Neurobiol., Univ. of Vermont, Burlington, VT, 05405.

Increased sympathetic nerve activity in the spontaneously hypertensive rat (SHR) may be partially due to alteration of the membrane properties of postganglionic neurons. Since the SHR also exhibits significant behavioral hypertensive response to stress, the relationship of neuronal alterations in SHR to the hypertensive phenotype is uncertain. To determine whether the decreased accommodation observed in SHR ganglion cells (Yarowsky and Weinrich, Hypertension. 26, 255, '88) is associated with hypertension, we examined active and passive membrane properties of cells in four inbred rats (WKY, SHR, Wistar-Kyoto, and WKAH). The SHR is both hypertensive and hyperactive; WKY is neither. The WKHA is hypertensive; the WKHT is hypertensive, but not hypertensive.

Membrane properties were examined in 24 WKY and 40 WKHA superior cervical ganglia cells. No significant differences were observed in resting membrane potential, input resistance, the after hyperpolarization, and transient outward current for action potentials generated among the four strains. In contrast, the number of action potentials generated during a 1 nA, 400 msec depolarizing pulse was significantly greater in both hypertensive strains compared with WKY and WKHA and less in WKY. The WKY (2.9 ± 0.56); the WKHA ganglion cells generated an intermediate number of action potentials (3.6 ± 0.63). The decreased ganglion cell accommodation correlates with an overall decrease in the number of neurons in each population that exhibited a non-phaic response (±4 action potentials) to the 400 msec stimulus: 21% in the WKY, 26% in the WKHA, 32% in the SHR and 48% in the WKHT.

559.8


The major aim of the present investigation was to determine whether sympathetic components of peripheral nerves (PN) were modulated by cardiac- or respiratory-related vagal afferent and/or chemoceptor input. In Saffan-anesthetized, paralyzed and artificially ventilated (100% O2) newborn piglets aged 1-40 days, different branches of PN were recorded along with CS phrenic (PHR) root activity, A0, EKG, and EEG. Age-related differences were observed for both types of modulation which were independent of vagal afferent inputs. Piglets < 1 wk old exhibited very little spontaneous PN activity related to either cardiac or respiratory rhythms. However, respiratory-related activity in PN increased with age in older piglets. Cardiac modulation by respiratory rhythm was revealed by coherence spectral analyses as indicated by significant coherence estimates over the 3-5 Hz range; such coherence was stronger in older piglets. Hypoxia significantly increased coherence regardless of age. The results indicate that cardiac and respiratory modulation of postganglionic sympathetic activity is delayed postnataally compared to preganglionic recordings. (Gootman et al. Am. J. Physiol. 1991; 261:R147). (Supported by NIH grant HD-28931).

559.10

INSPIRATORY COMPONENT OF SPLANCHIC SYMPATHETIC NERVE ACTIVITY IS RESISTANT TO THE SYMPATHOLYTIC DRUG CLONIDINE. N. Koshiya* and P.G. Grover, Dept. of Pharmacology, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

Splanchnic sympathetic nerve (SN) and phrenic nerve discharges (PND), and arterial pressure (AP) were recorded in rats that were either vagotomized, aortic depressor/paralyzed, and artificially ventilated Sprague-Dawley rats (n=7). SNP and PND were rectified, analog integrated at 30 ms retset-interval and digitized. Distribution of the SNP in the central respiratory cyclus was analyzed by mean of PND burst-triggered averaging. During control period, mean AP was 106±10 mmHg (mean±SEM). SNP distributed throughout the respiratory cycle with increased activity in inspiratory/early-inspiratory phase and peak activity in the post-inspiratory phase. Splanchnic nerve was given i.v. at small (15-20 μg/kg, n=6) and large doses (200-250 μg/kg, n=7). Small dose of clonidine produced brief hypotension (<30 sec, 150±20 mmHg peak) followed by moderate hypotension (80±20 mmHg) which was accompanied by a reduction in SNP (<50% control value). Large dose of clonidine produced sustained hypertension (>10 min, 137±33 mmHg) accompanied by silence of SNP. During the sustained hypertension, the vasocilatator sodium nitroprusside was administered i.v. (80-150 μg/kg). The nitroprusside-induced hypotension retrieved a component of SNP which was barosensitive but resistant to clonidine. During those hypotensive periods (both after small dose of clonidine and during nitroprusside-induced hypertension after large dose of clonidine), SNP was largely attenuated in respiratory phase and, in most cases (6 out of 7), SNP showed single inspiratory peak with very small activities in the post-inspiratory/expiratory period. In 2 cases, despite the substantial decrease in total SNP, the amplitude of the remaining inspiratory peak was virtually unchanged from the control level. These results suggest that the sympatholytic drug clonidine works on the sympathetic nervous system throughout the respiratory cycle and, the central inspiratory-related component of SNP is least sensitive to the drug.
559.13


Increasing evidence suggests that parasympathetic ganglia are sites of complex synaptic integration rather than simple relay stations. The present study was done to establish whether direct afferent input to parasympathetic neurons of mammalian ganglia may provide another mode of synaptic modulation. Whole mount preparations from the guinea pig cardiac were utilized to localize afferent inputs by immunohistochemistry.(1) The results of these studies are reviewed. Immunohistochemical analysis demonstrated that most parasympathetic neurones were surrounded by fibers immunoreactive for both substance P (SP) and CGRP. Intracellular recordings were obtained from individual parasympathetic neurones maintained in an oxygenated Krebs solution at 37°C. Action potentials could be elicited both by intracellular current injection and stimulation of interganglionic fiber bundles. In some cells, high frequency (1-10 Hz) interganglionic fiber stimulation produced a slowly developing depolarization of 4-10 mV which lasted for 5-30 sec. These depolarizing events were unchanged in the presence of 100 μM benzamidinium and 1 μM atropine but were significantly diminished by superfusion with calcium chelate solution. A prolonged depolarization was also produced by local application of capsaicin (1 μM), which releases both SP and CGRP from afferent terminals. Direct application of either SP or CGRP (100 μM) by pressure injection depolarized these neurones. This peptide-induced depolarization was often associated with a decrease in input resistance and an increase in excitability. We suggest that the slowly developing depolarization produced by interganglionic fiber stimulation is due to the release of SP and/or CGRP from afferent terminals. Further, we suggest that afferent input to the cardiac ganglia may be involved in local reflex modulation of the parasympathetic cardiac neurones. Supported by NIH grants NS 26995, DK 45410, and NS 23978.

559.15

GUANETHIDINE: EVOKES Vasodilatation IN MESENTERIC ARTERIES BY ACTING ON SENSORY NERVES. Z.-I. Zheng, K. Shimamura, T.L. Anthony and D.L. Krueger*. Department of Physiology, West Virginia University, Morgantown, WV, 26506.

Capsaicin-sensitive sensory nerves mediate an endothelium-independent, NANC vasodilatation in guinea-pig mesenteric arteries. This vasodilatation is potentiated by previous administration of guanethidine (30 μM) presumably through depletion of vasoconstrictor neurotransmitters from sympathetic nerves. Our current hypothesis is that guanethidine also releases transmitter(s) from capsaicin-sensitive sensory nerve and thereby invokes vasodilatation. To test this hypothesis, segments of endothelium-denuded mesenteric arteries (5.8 mm long, 200 μm wide) were suspended in tissue bath (50 ml) containing normal Krebs solution and stretched to L0. Each vessel was precontracted with methoxamine (30 μM). Administration of sodium nitroprusside evoked a 100% relaxation of these vessels. Administration of guanethidine (30 μM) evoked a 72±5% relaxation of the precontracted vessels. Pretreatment with capsaicin (10 μM) reduced the guanethidine-induced vasodilatations to 22±7%. The NOLA-mediated effects could be almost completely reversed with hydroxyquinidine (HQQ, 10 μM), an intermediate of nitric oxide, but not L-arginine (1 mM). To determine whether this effect of guanethidine on sensory nerves was dependent on transport, the vessels were pretreated with cocaine (5 μM) for 5 min or ouabain (25 μM) for 15 min. Cocaine decreased the guanethidine-induced vasodilatations to 35±14%. Likewise, ouabain diminished the guanethidine-induced vasodilatations to 35±14%. However, cocaine, diminished the guanethidine-induced vasodilatation to 9±5%. The guanethidine vasodilatation was not affected by propranolol (100 μM), 6-hydroxydopamine (6-OHDA, 30μM) or tetraodoxidine (TTX, 10 μM). Support: NIH-NS 27781.

560.1

POSSIBLE ROLE OF THE FUNDUS STRIATA VP RECEPTORS IN BLOOD PRESSURE (BP) CONTROL. P. Krnac, M. K. Cooper* and G. J. Pitkanen. Neuroscience Research Group, University of Calgary, Calgary, Alberta, T2N 4N1, CANADA.

Vasopressin, given intracerebrally to rats, can induce motor disturbances, increases in BP and antipyresis. The possibility that highly concentrated vasopressin binding sites in the fundus striati (FStR) could participate in these effects was investigated using chronically implanted cannulas aimed at this area. A unilateral bilateral injection in the FStR of 100μg/μl VP did not induce motor disturbances (scored visually) after the first and even the second injection 24 hours later. This body temperature recorded simultaneously (i.p. minitnter) was not altered by the VP injections. After a bilateral injection of 100μg/μl/25μl VP in the FStR, the fever induced by an i.p. injection of the lipopolysaccharide of X. Coll (50μg/kg) or by interleukin 1β (0.1μg/kg) was not affected. In contrast, a bilateral injection of 100μg/ μl/25μl VP into the ventral tegmental area (VTA), induced an increase in BP (19.1±2.24 mmHg, p<0.001) compared with saline controls while the heart rate was not modified (p>0.4). This increase in BP was blocked by a V1 antagonist; OT and a V2 agonist were ineffective.

This study suggests then that the VP receptors in the FStR could intervene in the regulation of the BP together with other regions known to be implicated in such a role.

560.2

AUTONOMIC STRUCTURES IN RAT BRAIN SHOW INCREASED NUMBERS OF C-FOS POSITIVE CELLS DURING CLONIDINE WITHDRAWAL. B.L. Stennett, M.E. Fisher and P.G. Guzelsu*. Department of Pharmacology, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

Animals and humans become dependent on clonidine (clon) following chronic administration of this α₂-adrenergic receptor (α2) agonist as revealed by either spontaneous or antagonist induced behavioral and autonomic withdrawal symptoms. The present study sought to determine whether specific autonomic areas of the brain would exhibit increased c-fos mRNA during clon withdrawal to the presence of c-fos nuclear protein as an indication of cell activation. Seven male Sprague-Dawley rats were briefly anesthetized with ether and implanted subcutaneously in the back of the neck with Alzet osmotic minipumps containing either 200μg/h clon or saline. After 5 days, these animals were returned to their home cages and after 7-10 days were injected i.p. with either saline or the α2 antagonist yohimbine (5μg/kg) and 2 hours later were deeply anesthetized with Nembutal (i.p. and perfused with 4% paraformaldehyde). Brain tissue was cut in the frontal plane and sectioned on a cryostat in 20 μm sections. Sections were incubated in c-fos antibody (Santa Cruz) and reacted for immunoreactivity using a standard avidin-biotin complex with VIP as the coimmunostaining substrate (Vector). Rats were run in the experimental protocol in 4 groups of 4 or 5. The groups consisted of 3 control rats (sal-sal, sal-clon-sal) and 1 or 2 experimental rats (clon-sal).

All experimental rats showed behavioral symptoms of withdrawal such as wet shaking, paw shakings and increased locomotion and escape behavior while none of the control rats exhibited these behaviors. An increase in the c-fos mRNA expression in the clon+ at withdrawal (clon) was noted in many brainstem autonomic structures including rostral ventrolateral medulla (RVLM) and raphe nuclei. Brainstem neuronal c-fos mRNA expression was expressed with the following symbols:

- This increase was quantified for RVLM, (R1.6 6 4 4 cells/sec in section co/col 3 5.0±0.8 in clon/sal, 8.2±1.0 in sal/sal and 7.3±1.4 in sal/sal) and LC (22 884.0 cells/sec in clon+ at 3 8.5±0.5 in sal/sal, 4.8±0.7 in sal/sal and 4.4±0.7 in sal/sal).
- The increase in neuronal activity in brainstem autonomic areas could explain the rebound hyperactivity observed during clonidine withdrawal.

Supported by NIH Grant DA07353.
60.3 CARDIOVASCULAR EFFECTS ELICITED BY MICROINJECTIONS OF NEUROPEPTIDE FF (NPPF, MORPHINE MODULATORY PEPTIDE) IN THE NUCLEUS TRACTUS SOLITARIUS. J.H. "Jhamandas", K. Harris and K.H. "Kanagasabapathy. Department of Medicine (Neurology) & Division of Neuroscience, University of Alberta, Edmonton, Alberta and Department of Pharmacology & Toxicology, Queen’s University, Kingston, Ontario, Canada.

Nucleus tractus solitarius (NTS), termination site of baroreceptor afferents is enriched in both NPPF immunoactivity and binding sites. The function of NPPF in this area is unclear. We have examined the potential of NPPF to influence blood pressure in the urethane anesthetized rat after microinjection of the peptide in the NTS. Unilateral injections of NPPF (0.2-1.0 nmol, 50-150 nl) produced elevation in blood pressure. The peptide induced hypertension was of a finite latency and outlasted the injection. Following apressor response, higher doses of peptide had to be injected to produce a similar response, suggesting a desensitization to its action. We also observed similar profile of pressor responses following unilateral injections of the NPPF analog (IDME)YBaFFa (0.2-1.0 nmol, 50-150 nl) within the NTS. Naloxone (2-4 mg/kg) administered intravenously failed to influence the effect of NPPF analog. Sites within the NTS where NPPF elicited the most consistent effect included medial and dorsomedial NTS at and just rostral to the level of area postrema. These results suggest that in the NTS, NPPF may serve to modulate cardiovascular function.

Supported by the Medical Research Council of Canada.

60.5 CHARACTERIZATION OF BRAINSTEM CIRCUITS FOLLOWING ELECTRICAL STIMULATION OF THE CENTRAL NUCLEUS OF THE AMYGDALA (CNA): A COMBINED C-FOS AND RETROGRADE TRACING STUDY. T. Petrow, J.H. "Jhamandas", T. Klotz, Department of Anatomy and Cell Biology and Medicine (Neurology), Univ. of Alberta, Edmonton, Alberta, Canada.

We have previously shown that electrical stimulation of the CNA induces expression of Fos (the product of the immediate early gene c-fos) in catecholaminergic (CA) neurons in the ventrolateral medulla (VLM) and the nucleus of the solitary tract (NTS). In this study we electrically stimulated the CNA and examined: 1) the connectivity of activated VLM neurons with the NTS; 2) the connectivity of activated NTS neurons with the VLM; 3) the proportion of activated CA neurons that project to the VLM or NTS respectively.

Rhodamine-labelled latex microspheres (RLL-LLM) were stereotaxically injected into the NTS; 4 days later, the ipsilateral CNA was electically stimulated for 60 min (20-50 mA, 10 sec pulse trains, 50 Hz, 100 pulses/sec) under urethane anaesthesia. In control animals the electrode was positioned in the CNA without delivery of current. Brains were then processed for Fos and toxine hydroxyroxyl (TH) immunocytochemistry. In the experimental animals increased number of profiles that contained RLL-LLM were Fos immunoreactive (IR) in the ventrolateral VLM and NTS. 1) In the VLM 30% of the Fos-IR cells projected to the NTS. 2) In the NTS 12% of the Fos-IR cells projected to the VLM. 3) Of the CNA activated, RLL-LLM containing neurons, 17% in the VLM and 2% in the NTS were also TH-IR.

The results indicate that local brainstem circuits between the VLM and NTS may be involved in mediating CNA-induced activation of medullary neurons. CA and non-CA autonomic cells may participate in a reciprocally activated NTS and VLM neurons.

Supported by MRC and AHFMR.

60.6 ALTERATION OF THE CARDIAC BAROREFLEX APPEARS EARLY DURING PREGNANCY IN CONSCIOUS RABBITS. V.L. Brook*, and R.R. Quenell, Dept. of Physiology, Oregon Health Sciences University, Portland, OR 97201.

There is evidence that reflex increases in heart rate (HR) and other effectors are reduced during pregnancy. The present study tested the hypothesis that the change in baroreflex occurs early in pregnancy, coincident with increases in the steroid hormones estrogen and progesterone. Rabbits were treated with either estradiol valerate and vena cava catheters. When the animals were nonpregnant (n=8), the relationship between arterial pressure (BP) and HR was determined by first decreasing BP with infusion of increasing doses of nitroprusside (3.6,12.24,48 gg/kg·min)and then by increasing BP with phenylephrine (0.5,1,2,4 mg/kg·min). The median baroreflex was reassessed in the same animals (n=3-7) after about 1,2,3 and 4 wk of pregnancy (term is 31 days). Differences in the baroreflex curves between pregnant and nonpregnant rabbits were determined by comparing with ANOVA parameters generated by logistic analysis. BP was 65±2 mmHg in nonpregnant rabbits and was not significantly different in pregnant rabbits. HR increased (p<0.01) from 142±2 to 174±6 bpm after 3 and 4 wk of pregnancy. Baroreflex relationships between BP and HR were not altered after 1, 2 and 3 wk of pregnancy. However, maximal reflex gain decreased from 28.8±5 to 6.1±4 bpm/mmHg after 4 wk of pregnancy (p<0.01). These results indicate that the cardiac baroreflex of pregnancy, and support that increases in estrogen and progesterone do not mediate the changes that occur. Supported by NIH Grant HL 39923 and MRF of Oregon.

60.7 COMPARISON OF HEMORRHAGE- AND NITROPRESSURE-INDUCED Fos EXPRESSION IN BRAINSTEM NEURONS THAT PROJECT TO THE PARAVASCULAR CEREBRAL NUCLEUS (PVN). K.H. "Harriss", D. MacTavish, T.L. Krztof and J.H. "Jhamandas", Depts. of Medicine (Neurology), Anatomy & Cell Biology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2B7

We previously reported that hemorrhage-induced hypotension activates neurons in the nucleus of the solitary tract (NTS) and ventrolateral medulla (VLM) that project to the PVN. In the present study, Fos-immunoreactivity (FI) was used to compare the activation of PVN-projecting brainstem neurons in response to hemorrhage and sodium nitroprusside (SN)-induced hypotension. Under anesthesia, rats received injections of rhodamine-labelled latex microspheres into the PVN and were instrumented with arterial and venous cannulae. One week later, conscious rats (n=6) were hemorrhaged (5-6 ml) to lower and maintain arterial pressure (AP) to 30-50% of resting AP for 1.5 h. A separate group (n=6) received SN intravenously (4 mg/kg,0.5 ml) to lower and maintain the AP to 30-50% of baseline for 1.5 h. A comparable number of neurons with FI were observed at all levels of the NTS and VLM following both hypotensive stimuli. The only exception was the NTS, at the level of area postrema, where greater FI was found following hemorrhagic hypotension. In sham, controls, few neurons with FI were found. At all levels of the NTS, SN-induced hypotension led to FI in a significantly greater number of retrogradely labelled cells (40-50%) than in hemorrhaged animals (16-30%). These results show that both hemorrhage- and SN-induced hypotension activate medullary neurons in the NTS and VLM which project to the PVN. Moreover, quantitative analysis of FI following the two hypotensive stimuli suggests that a change in blood volume accompanying hemorrhage is not a significant factor in the activation of NTS and VLM neurons.

Supported by the MRC and Heart & Stroke Foundation of Canada.

60.8 ACUTE BLOOD PRESSURE CHANGE ELICITS HIPPOCAMPAL THETA RHYTHM AND BEHAVIOURAL AROUSAL IN THE UNRESTRAINED CONSCIOUS RABBIT. Jing-Hu Tan* and W.W. Bloss, Centre for Neuroscience, Flinders University, Bedford Park, SA 5042, Australia.

Acute changes in arterial blood pressure (AP) cause arousal from sleep in lambs (Fowell and Johnson, Brain Res. 121 (1977) 154) and monkeys (Krukoff et al., Physiol. (256) 1989 H434), a response mediated via baroreceptor afferents. arousal is accompanied by desynchronisation of the neocortical EEG, but no studies have reported the effect of blood pressure changes on hippocampal theta rhythm. In tri-geminal operations in New Zealand White rabbits (3-2 kg), anaesthetised with 1% halothane, we implanted micro-tip bipolar EEG electrodes in the skull and the lateral hippocampus, a capsule occluded around the thoracic inferior vena cava, and cannulae in an external jugular vein and a common carotid artery. The lines were kept open by constant infusion of heparinised saline. The rabbit remained in a cage on a swivel on the roof for freely accessing electric and fluid channels. The EEG and AP signals were recorded with MacLab (AD Instruments). The rabbit's behavior was recorded with a video camera.

The hippocampus was divided into 3 regions: dorsal, middle, and ventral. EEG in the dorsal area was reduced by inflation of the IV cuff, and increased by phenylephrine (3-10 ?g/kg i.v.). Changing AP beyond a threshold (approx 60 mmHg) in either direction caused hippocampal theta rhythm (7-10 Hz), sometimes followed by behavioral arousal. Our data suggest that major alteration in baroreceptor inputs affects arousal state.


Nitroglycerin (NTG) is a well known vasodilator, commonly used in the treatment of angina pectoris. A less familiar, but very intriguing clinical application of NTG is the diagnosis of primary vascular headache, as it consistently induces a spontaneous-like migraine attack in predisposed subjects. Recent findings have shown that NTG is capable of inducing the release of different neurotransmitters, such as norepinephrine and calcitonin-gene-related peptide, in the central nervous system (CNS). These findings have suggested that NTG may act, at least partially, through a centrally-mediated mechanism, rather than as a pure direct vasodilator. The prote-cytochrome c-oxidase encoded by a nuclear protein Fox, whose expression in certain brain regions can be induced by a variety of stimuli. For this reason, the immunohistochemical analysis of Fox-like immunoreactivity has been proposed as a highly sensitive method for the mapping of signaling pathways in the brain. Moreover, Fox contributes to the formation of the protein activator AP-1, which, in turn, triggers the transcription of specific downstream genes, ultimately cooperating in the regulation of target genes that may undergo cellular adaptive responses.

The aim of the present study was to evaluate, in the rat, the possible CNS effects of systemic NTG by means of the induction of c-fos activation and AP-1 expression. The activation of c-fos was evaluated by immunocytochemical detection of Fox-like immunoreactive neurons, while AP-1 expression was detected by electromobility shift assay (EMSAs). The results obtained by a time course study and immunocytochemical analysis reveal the existence of significant differences in both the localization and number of positive neuronal elements expressing c-fos in brain areas submitted to NTG injection demonstrate AP-1 expression as early as the second hour post-administration. These data strongly suggest that NTG can induce a selective activation of specific CNS nuclei (supported by NIH grant NS213323)
560.9 MORPHOLOGY OF SYMPATHETIC GANGLION NEURONS IN SPONTANEOUSLY HYPERTENSIVE RATS. C. Cameron* and K.G. Reit, Department of Anatomy and Cell Biology, University of North Dakota School of Medicine, Grand Forks, ND 58202.

Increased levels in nerve growth factor (NGF) in the walls of muscular arteries has been hypothesized as a contributing factor in the development of hypertension in spontaneously hypertensive rats (SHR). Our previous studies of the trophic effects of NGF on sympathetic ganglion neurons prompted us to hypothesize that significant changes in components of the autonomic nervous system may also lead to the development of hypertension. Superior cervical ganglion (SCG) neurons in 6-7 week-old SHR and normotensive Wistar-Kyoto (WKY) rats were intracranially filled with a 5% solution of Lucifer yellow in order to visualize the dendritic arborizations of these neurons. Each intracranially filled neuron was drawn by camera lucida, and cell body size, total dendritic length, and number of primary dendrites were measured and compared between groups. Preliminary experiments show that although SHR ganglion neurons tend to be larger and have greater total dendritic lengths, the differences were not statistically significant. In addition, the number of primary dendrites elaborated by these neurons did not differ. An interesting observation we have made in SHR animals, however, is that there is extensive dye-coupling between neurons of the SCG not seen in the WKYs. Previous investigators have shown that there is enhanced synaptic transmission through the SCG of the SHR. Our present evidence suggests that this may not be the result of large-scale changes in SCG neurons and preganglionic innervation but is possibly a result of the establishment of local ganglionic electrical connections.


Somatovisceral reflex arcs mediate cardiopulmonary adjustments to pain and exercise. The structural underpinnings of short intraspinal reflex pathways were examined using intraspinal transport techniques in chloral hydrate (0.5mg/kg)-anesthetized adult and 8-12 day-old neonatal Sprague-Dawley rats. Study 1. Do first order axial muscle afferents project to spinal autonomic ganglia? Ten T1 dermatomes (DLY) were injection labeled using the intrasegmental injection of D-Lucifer Yellow (DLY) were slowly injected, bilaterally, into the lenticulissimus muscle. Following 8-9 day survival periods, thoracolumbar spinal segments were sectioned and processed immunocytochemically for DLY. Transganglionic transport of DLY labeled first order muscle afferents in the thoricospinal cord served as painful; physiological responses of the intermediolateral column (ML) in mature (n=8) and young (n=3) animals. Study 2. Do neurons in the dorsal horn project to autonomic motor nuclei? Preganglionic deposits of Phascolus vulgaris leucoagulatum (n=13) centered on lateral loci in upper thoracic (T1-2) or thoraco-lumbar (T11-12 or L1-2) spinal segments transported, intraesomally, to the ML and nucleus intercalatus. A larger medial fiber trajectory was descripted in the dorsal gray commissure and terminated within a mirror locus in the dorsal horn on the contralateral side. Deposits centered to medial loci in the dorsal horn harned in laminae VII and X and the ventral horn, bypassing the ML. We conclude: Intraspinal-autonomic circuits may provide an anatomical substrate for the early reflex component of the exercise pressor reflex. The direct spinhal-autonomic reflex arc may be reinforced by this way of a multiyamic communica trial pathway. (Supported by a 5-year grant to the National Cancer Institute of Canada.)

560.13 TRIGEMINALLY MEDIATED ALTERATIONS OF NORMAL CARDIO-RESPIRATORY RHYTHMS DURING TRANSSANAL APPLICATION OF CARBON DIOXIDE. F. Yavari, W.M. Panetton, and J.H. Harting*, Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Psychophysiological studies in humans have shown that nasal stimulation with carbon dioxide (CO₂) induces reflex changes. Similarly, studies in the cat have shown that such effects may be mediated through the medullary dorsal horn. Since the medullary dorsal horn is also a probable site for the mediation of the "diving response", we have studied the cardiorespiratory responses in the rat which result from applying CO₂ across the nasal mucosa. The animals were anesthetized with intraperitoneal injections of a mixture of chloral-urethane. Cannulas were inserted into the femoral vein and artery for the delivery of drugs and recording of blood pressure, respectively. Other tubing was placed in the trachea for breathing and into the nasopharynx for drawing air over the nasal mucosa. The stimulus was 10% CO₂ applied to the nose and drawn through the nose by gentle suction applied to the nasopharyngeal tube. Such stimulation caused an abrupt drop in heart rate, and in the majority of the experiments, apnea and an elevation of blood pressure. In other experiments, CO₂ caused deep breathing and hyperventilation (no apnea) or arhythmic breathing with apneic periods. The bradycardia was not the result of activating baroreceptors since it persisted even in those cases where an alpha-1 adrenergic receptor blocker, was used to prevent the increase in blood pressure. The CO₂-induced bradycardia was vagally mediated since it could be completely abolished after intravenous atropine. The nasal application of CO₂ also induces a blockade of the bradycardia and hyperventilation, suggesting that the responses are trigeminal mediated. These CO₂ induced responses previously have not been reported and resemble those of the diving response. Supported by NIH grant HL3471.
560.15 CARDIOVASCULAR EFFECTS OF NITRIC OXIDE ON BRAINSTEM NUCLEI OF RATS. C. J. Tong,* H. Y. Liu, H. C. Lin and C. S. Jung, Dept. of Medical Education and Research, Kaohsiung Veteran General Hospital, Kaohsiung, Taiwan, R.O.C.

Nitric oxide (NO) has been reported as a second messenger and neuromodulator in vivo. In this study, we evaluated the cardiovascular effects of NO in the nucleus tractus solitarius (NTS), rostral ventrolateral medulla (RVM) and area postrema (AP) of male Sprague-Dawley (SD), Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) respectively. Unilateral microinjection (60 nl) of L-arginine (L-Arg, 1-100 mmol) into the NTS and RVM produced a prominent dose-related decrease in heart rate and blood pressure. The depressor and bradycardic effect of L-Arg were significantly attenuated by the pretreatment of NO synthase inhibitor -Nω- monomethyl-L-arginine (L-NMMA). The depressor effect of L-Arg in the NTS of SHR was less sensitive than the WKY. Furthermore, the cardiovascular effects of L-Arg was potentiated via intravenous injection of the lipoxygencerase inhibitor (LPS). In addition, a simultaneously injection of L-Arg and L-NMMA was noted during the period of L-Arg induced depressor effect in the NTS. However, there is no significant cardiovascular effect of L-Arg in the AP. These results suggest that NO is involved in the central cardiovascular regulation. The depressor effect of L-Arg in the NTS might be through the inhibition of sympathetic nerve activity.

560.17 PHARMACOLOGICAL CHARACTERIZATION OF THE 10 HZ SYMPATHETIC NERVE DISCHARGE FREQUENCY. M. E. Clement* and R. F. McCall. The Upjohn Company, Kalamazoo, MI.

Most of the power in the discharge frequency of sympathetic nerve in baroreceptor denervated decerebrate or urethane-anesthetized cats concentrates in a 2-4 Hz band and a narrower frequency band around the 10 Hz range. Barman and Gebber have shown that chemical inactivation of the medulla medullary raphe with muscimol eliminates the 10 Hz peak, while stimulation of this area can induce the 10 Hz rhythm. Our laboratory has recently examined the effect of a number of pharmacological agents on the frequency distribution of sympathetic activity. We found that systemic administration of the GABA antagonist picrotxin (0.01-1 mg/kg) selectively suppressed the 10 Hz power band in a dose dependent manner. Incremental doses of picrotxin also tended to shift the 10 Hz peak to higher frequency. Low doses of 8-OH-DPAT (1-5 µg/kg) selectively inhibited the 10 Hz power band in a dose dependent manner. The inhibitory effects of 8-OH-DPAT could be reversed by the 5-HT antagonist piperperone. The putative 5-HT antagonist WAY100135 (0.01-0.1 mg/kg) did not reverse 8-OH-DPAT but did inhibit 10 Hz activity when given alone. The 5-HT antagonist methysergide (0.01-1 mg/kg) caused a dose dependent shift in the 10 Hz band to lower frequencies. Chlorimipramine, a 5-HT uptake inhibitor, also eliminated the 10 Hz peak in SND, but did not block stimulation induced 10 Hz activity. These data suggest that 5-HT and GABA mediate the level of excitability of the central oscillator responsible for the generation of the sympathetic 10 Hz frequency.

REGULATION OF AUTONOMIC FUNCTIONS: CNS PATHWAYS AND TRANSMITTERS


The nucleus of the solitary tract (NTS), dorsal motor nucleus of the vagus (DMNX), and area postrema (AP) are interrelated structures important in the modulation of visceral function, including those mechanisms underlying nausea and vomiting. Neuroleptics, probably acting on dopamine receptors, have potent antieptic actions. In order to establish and characterize the role of medullary dopamine receptor subtypes in the control of nausea and vomiting, we studied the distribution of D1-D5 receptors. Fresh frozen human brainstem sections from normal controls, n=5, sectioned at 20 µm and slide mounted, were incubated with [3H]apipiphrine for D2 and D3 receptors, [125I]epidepride for D2, D3, and D4 receptors, [3H] SCH23390 for D1 and D5 receptors, and [3H]nomipiphrine for D2, D3, and D4 receptors, and processed for autoradiography. Dopamine receptors were most densely distributed over the medial and intermediate subnuclei of the NTS and the DMNX. Moderate binding was noted over the lateral, intermediate, and substantia gelatinosa subnuclei. The medial region of the NTS receives a dense projection from the subdiaphragmatic vagus. Dopamine receptors in this region of the NTS, as well as in the DMNX, probably play a central role in antieptic action of neuroleptics.


The pairing of sensory and motor representation of a specific organ within certain levels of the avian nucleus of the solitary tract (NTS) and dorsal motor nucleus of the vagus (DMNX) could facilitate vago-vagal reflexes. The present study investigated the existence and peptide content of local NTS projections to DMNX that could subserve esophageal reflexes. Fluorescin-latex beads were injected into an anterior DMNX subnucleus, which innervates the esophagus. Retrogradely labeled NTS cells were observed ipsilaterally and contralaterally and were most dense in medial tier NTS subnuclei, which are recipients of vagal afferents from the alimentary tract. The combination of retrograde labeling and immunofluorescence techniques revealed many bombesin, enkephalin, and neurotensin immunoreactive medial tier NTS cells that were double-labeled. Many of the enkephalin and neurotensin containing double-labeled cells were located in medial tier NTS subnuclei that are the major recipients of esophageal afferents and thereby could subserve as interneurons in esophageal reflexes. Bombesin double-labeled cells were found in medial tier NTS subnuclei that are major targets of gastric afferents. These bombesin containing cells project to the anterior DMNX may serve to integrate esophageal and gastric functions. On-going experiments will attempt to verify the axonal projections of these medial tier NTS cell groups to esophageal DMNX motoneurons by combining anterograd axonal tracing with retrograde labeling techniques. Support NSF R11-B922106.
561.3 DISTRIBUTION OF BOMBESIN-LIKE IMMUNOREACTIVITY IN THE NUCLEUS OF THE SOLITARY TRACT (NTS) AND DORSAL MOTOR NUCLEUS (DMN) IN HUMANS. R.B. Lynn1*, R.R. Miselis2*, and T. Hori3* 1Dept. of Med., Thomas Jefferson Univ., Philadelphia, PA 19107; 2Dept. of Animal Biol., Univ. of Penn., Philadelphia, PA 19104; 3Clin. Brain Disorders Branch, NIMH/RP, Wash., D.C. 20032 Bombesin, also referred to as gastrin releasing peptide (GRP-14-27), has important autonomic and behavioral effects when injected into the NTS/DMN complex in rats. Bombesin-like immunoreactive (Bb-LI) labeling is visible in the rat NTS/DMN complex but has not been described in the human medulla. Four normal human medullas were flash frozen and subsequently thawed and fixed. Frozen sections (40μm) through the obex, diencephalic, and mesencephalic subnuclei of the NTS and the dorsal part of the DMN. The substantia gelatinosa and the tracts of the NTS appeared void of Bb-LI staining. Rare Bb-LI labeled cell bodies were scattered within the NTS. Bb-LI labeling was also noted in the A1 region and the spinal trigeminal nucleus. In controls, all Bb-LI staining was abolished by preabsorption of the primary antisem with bombesin peptide. This distribution of labeling is very similar to that of labeling in the non-colchicine treated rat. The anatomic similarities between human and rat suggest that bombesin has similar functions in the visceral neurex of these two species. Supported by KO8 DK02094(1); GM27739(2); IRP-NIMH(3).

561.5 GLUTAMATE-IMMUNOREACTIVE NEURONES AND AXON TERMINALS OF THE NUCLEUS TRACTUS SOLITARIUS (NTS): DEMONSTRATION OF GLUTAMATE IN VAGAL SENSORY APPARENCES. R.M. Sykes, D. Jordans* and P. N. Izzo Dept. Physiology, Royal Free Hosp. School, Brookfield Hill St., London, NW3 2PF, U.K. Although there is a growing body of literature regarding the actions of glutamate in the NTS very little is known about the anatomy and organization of the putative glutamatergic neurones in this region. We have used post-embedding immunocytochemical techniques glutamate-containing neurones to be examined at both the light and electron microscopic level. Furthermore, by combining this technique with anterograde labelling this study begins to examine the origins of glutamate-containing neurones observed in the NTS. Light microscopic examination of semi-thin plastic sections revealed glutamate-immunoreactive neurones throughout the NTS and gracile nucleus with a distinctive organization at intermediate levels where groups of immunoreactive perikarya were observed. Axonal boutons were also labelled. These were found through to the subnucleus, providing a very dense and apparently homogeneous innervation.

Ultrastructural examination of glutamate-immunogold labelled sections identified a population of axonal boutons which displayed common morphological features and formed exclusively asymmetric synaptic specializations. The major post-synaptic targets of these boutons were small to medium-sized dendrites. Glutamate immunocytochemistry of sections containing anterograde labelling following the injection of horseradish peroxidase into the nodose ganglion revealed that these axons contribute to the population of glutamate-immunoreactive axonal boutons in the NTS. Supported by the Wellcome Trust and British Heart Foundation.

561.7 TRANSYNAPTIC DOUBLE LABELING WITHIN THE VISCERAL NEUROAXIS WITH UNIQUELY MARKED STRAINS OF PSEUDORABIES VIRUS (PRV) INTO THE RAT PHARYNX. X. Rao, Z. B. Wiedner, and S.M. Allbritton Children's Hospital of Philadelphia, Univ. of Pa. Sch. of Med., Philadelphia, PA 19104. The initiation and control of swallowing is dependent on pre-oromotor neurons (POMNs) located primarily in NTS. The subnuclear location and connectivity of POMNs innervating the pharynx were determined using anterograde and retrograde tracing of PRV in 15 rats, PRV injections were made into the pharynx. Following a 48-96 h survival, brain sections were processed immunocytochemically for PRV. Anterograde labeling was limited to the semicompact formation (SC) of the NTS for a survival time of 48 h. At 56-62 h survivals, SC, PL, and PH were localized to the interstitial (IST) and intermediate (INT) subnuclei of NTS. At longer survivals, neuronal transections were observed in the INT subnuclei of the NTS, raphe nucleus, A5, hypothalamus, and forebrain. Pharyngeal POMNs are localized to the ist/P2uf and int/P2uf, the sites of termination of superior laryngeal nerve afferents, and have direct synaptic contact with spinal motoneurons. The pattern of labeling at longer survivals suggests widespread CNS control over pharyngeal motility. Supported by NIH grant DK-44487.

561.8 SYMPATHETIC NERVE FIBERS IN THE SUBDIAPHRAGMATIC VAGUS NERVE OF THE RAT SEEN WITH FLUOROGOLD, CT-HPR OR PSEUDORABIES VIRUS. M.Yang*, X. Zhao and R.E. Miselis Animal Biology, Philadelphia, PA 19104. To identify the source and number of the entry of sympathetic nerve fibers in the vagus nerve one of three retrograde tracers was injected into the subdiaphragmatic vagus trunk of the rat. We examined the upper sympathetic ganglia, the nodose ganglion, and the dorsal motor nucleus of the vagus (DMV) in the same animals. We find a small number of labeled sympathetic neurons at the cervical (C1-C6), thoracic (T1-T5), lumbar (L1-L5) and sacral (S2-S4) levels. All labeled sympathetic preganglionic fibers arising from the intermediolateral nucleus of the spinal cord via the intermediolateral cell column project to the superior cervical ganglion (SCG), which projects to the superior cervical ganglion (SCG), 30 in the coccidophagous region (CTR) and 25 in the other thoracic region (TR). The retrograde tracers were injected into the right vagus nerve of the rat. We found that the sympathetic preganglionic fibers originating from the intermediolateral nucleus of the spinal cord project to the superior cervical ganglion (SCG), which contains 30 of these neurons. All labeled sympathetic preganglionic fibers arising from the intermediolateral nucleus of the spinal cord project to the superior cervical ganglion (SCG), which contains 30 of these neurons. We found that the sympathetic preganglionic fibers originating from the intermediolateral nucleus of the spinal cord project to the superior cervical ganglion (SCG), which contains 30 of these neurons. All labeled sympathetic preganglionic fibers arising from the intermediolateral nucleus of the spinal cord project to the superior cervical ganglion (SCG), which contains 30 of these neurons. We found that the sympathetic preganglionic fibers originating from the intermediolateral nucleus of the spinal cord project to the superior cervical ganglion (SCG), which contains 30 of these neurons.

Recent evidence suggests that the hypothalamus may play an important role in stress-related gastrointestinal disorders. Stimulation of the paraventricular nucleus of the hypothalamus (PVH) can induce gastric efferent and motility changes that can be eliminated by vagotomy, suggesting that the PVH projects to the dorsal motor nucleus of the vagus (DMNV). Little is known, however, about the potentially important PVH influence on DMNV neurons. To address this issue, we used glass microelectrodes containing 2% Neurobiotin to record the responses of individual gastric- and intestine-sensitive DMNV neurons to PVH stimulation. The stomach and duodenum of 15 rats were separately cannulated to permit dissection with normal saline surrounding the subdiaphragmatic and gastric branches of the vagus nerve. Stimulating electrodes were also placed in the PVH. Following physiological characterization each neuron was injected with the tracer and reconstructed using the Eucentric Neuron Tracing System. A total of 18 DMNV neurons were successfully labeled. Most of these neurons were inhibited by intimal and/or gastric distension. PVH stimulation, however, inhibited 6 and excited 8 of the distention-sensitive DMNV cells (PVH stimulation did not affect the activity of the remaining 4 neurons). Interestingly, our data suggested that a given DMNV neuron’s response to PVH stimulation may be related to certain morphologic features. We found that neurons that were inhibited by PVH stimulation tended to exhibit a higher density of dendritic swellings than the neurons that were excited by this stimulus (p = 0.008). These preliminary results suggest that PVH may project to distinct subsets of gastrointestinal DMNV neurons. These two groups appear to receive similar input from the intestine, but opposing inputs from the hypothalamus. Supported in part by NS30083 and DC01074.

Pancreatic Polypeptide Increases the Activity of Dorsal Vagal Complex Neurons in vivo
Dana M. Mctigue* and Richard C. Rogers. Dept. of Physiology, College of Medicine, Ohio State University, Columbus, OH 43210.

Pancreatic polypeptide (PP), a hormone released during digestion, has been shown to alter vagal afferent traffic and elevate neurotransmitter levels in the dorsal motor nucleus of the vagus (DMNV). Little is known, however, about the potentially important PVH influence on DMNV neurons. To address this issue, we used glass microelectrodes containing 2% Neurobiotin to record the responses of individual gastric- and intestine-sensitive DMNV neurons to PVH stimulation. Cells within the DVC were identified in two ways: vagal stimulation to identify NTS vs DMN neurons or gastric inflation to identify On vs Off cells (neurons that increase or decrease firing rate during inflation, respectively). The results obtained indicate that over 65% of On cells and Off cells were stimulated by PP while none were inhibited. Furthermore, 60% of NTS cells were stimulated by PP. Preliminary studies examining DMN neurons demonstrate that 30% are inhibited while the rest are unaffected. These studies indicate that in general, gastric related DVC neurons are potentially stimulated by PP. When the entire population of DVC neurons is studied, more complex effects may be observed. The long-lasting stimulation of gastric related DVC neurons may provide a mechanism for the observed increases in gastric activity following PP administration (NS 30803 to RCR).

2-DEOXY-D-GLUCOSE (2DG) INDUCES C-FOS IN CENTRAL AND PERIPHERAL CATECHOLAMINE (CA) SYSTEMS. S. Ritter* and I. Llewellyn-Smith. College of Veterinary Medicine, Washington State University, Pullman, WA, U.S.A 99164, and Department of Medicine, Finders Medical Centre, Adelaide, S.A. 5042, Australia.

2DG is a glucose analogue that competitively inhibits glucose utilization. Responses to 2DG include increased feeding and adrenal medullary secretion and induction of Fos-like immunoreactivity (FLI) in the brain and adrenal medulla. Previous studies have shown that 2DG-induced feeding is impaired by lesion of brain CA systems and by pharmacological reduction of CA neurotransmission. In this experiment, 2DG was administered systemically and immunocytochemistry used to determine whether c-fos was induced in hindbrain neurons which express tyrosine hydroxylase (TH). Results show that 2DG induces FLI in some, but not all, TH-containing neurons in the A1/C1 and A2/C2 regions and in many neurons not containing TH. 2DG also induced FLI in a subpopulation of sympathetic preganglionic neurons retrogradely labelled by injection of the B subunit of cholera toxin into the adrenal medulla. Results identify specific subpopulations of brain CA and preganglionic neurons activated by glucoprivation.

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PP and PYY are circulating gut hormones that regulate digestion. Receptors for PP and PYY are present in rat gastric mucosa on both sides of the blood-brain barrier (BBB). When PP and PYY are microinjected into certain areas of the dorsal medulla inside the brain, they produce potent gastric effects. We hypothesized that PP and PYY are produced by cells within the CNS that project to areas with high densities of receptors, despite the failure to consistently demonstrate these peptides by radioligand receptor binding or immunocytochemistry. Objective: to determine if PP and PYY mRNA are present in brain, using RT-PCR Methods: Total RNA was extracted from the dorsal medulla and medulla of the rat brain, pancreas and distal 10 cm of ileum using the Trisolv method (Biocheck, Houston, TX). First strand cDNA was generated using a Moloney Murine Leukemia Virus reverse transcriptase reaction. Five ng of cDNA products were incubated with the PP or PYY primers and Taq DNA polymerase along with controls and standards for 35 cycles. The products were separated directly on an Nu-Sequagene agarose gel. The identity of the products was tested by Southern blot. Results: PP and PYY mRNA were detected in the dorsal medulla of the rat brain using the RT-PCR. The PP primers yielded a single band identical in size to the PP mRNA isolated from the pancreas. For PYY, two bands were amplified from brain mRNA, corresponding to predicted PYY mRNA fragments with a possible splice. The signal obtained for mRNA for PYY bands differed between brain and ileum. Southern blots labeled with 32P- or 32P-PYY cDNA probes demonstrated that the amplified PCR products hybridized with the appropriate cDNA. Conclusion: PP and PYY mRNA are present in the brainstem of rats. This supports the hypothesis that PP and PYY receptors in areas of the brain that are inaccessible to circulating hormones may have physiological roles.
561.15 REGULATION OF AUTONOMIC FUNCTIONS: CNS PATHWAYS AND TRANSMITTERS
THURSDAY AM


Previous studies have shown that the nucleus paragigantocellularis lateralis (PGi) strongly innervates the nucleus locus coeruleus (LC) and sympathetic nuclei of the spinal cord. However, other data suggest that PGi have not been systematically examined. In the present study, Phaeolus vulgaris-leucoglaucogmin (PHA-L) was used to comprehensively map PGi efferents in the rat. Multiple deposits of PHA-L were iontophoresed into the PGi. After 7-10 days, animals were perfused and processed for PHA-L immunostaining. Dense PHA-L terminal labeling was seen in the intermediolateral cell column and intermediate and lateral spinal cord segments, A1/C1 areas of the ventral medulla, the nucleus of the solitary tract, dorsal motor nucleus of the vagus (X), the hypoglossal nucleus (XII), the LC, medial and lateral parabrachial nuclei, Kollikier-Fuse nucleus, the paragigantocellular, the bilateral nucleus, the intermedio-dorsal nucleus and the intermediolateral nucleus. PGi and subcoeruleus ventralis nuclei were densely innervated by PGi, and a major divergence of these projections was observed. The present study shows that PGi efferents have a more extensive and complex pattern than previously reported. Further studies to identify the nature of these projections are currently underway.


The parabrachial (PB) and Kobliker-Fuse nuclei (KF) receive input from the somatosensory system, as well as from the efferent systems of the spinal cord. The present study was undertaken to define the somatosensory input to these nuclei. Our results demonstrated that the somatosensory system input to the PB/KF complex is more complex than previously thought. The somatosensory system input to the PB/KF complex is more complex than previously thought.


Electrolytic lesions of the lateral hypothalamus or the anteroverentral third ventricle reduces the salivary secretion induced by ICV injection of pilocarpine in rats. In the present study, we investigated the effect of septal (medial + lateral) electrolytic lesion (2 mA x 20 s) on the increase of the salivary secretion induced by ICV (lateral ventricle) injection of pilocarpine in anesthetized rats. The salivary flow was measured by pre-washed cotton balls inserted in the mouth. Twelve hours or more after the lesion (acute lesion) or 14 or more days (chronic lesion) after the lesion, the saliva was collected 1 min before (control) and 7 min after ICV injection of pilocarpine (120 mg/kg). ICV injection of pilocarpine in sham rats increases the salivary flow (420 ± 6 mg/7 min) compared to control (18 ± 5 mg/7 min). Septal lesion reduces (19± 2 mg/7 min; 287 ± 42 mg/min, respectively) the salivary effect of ICV pilocarpine, but not the basal salivary flow (26 ± 5 mg/7 min). These results suggest the septal lesions participates in the salivary secretion produced by ICV injection of pilocarpine in rats.

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Glucagon like peptide-1 (GLP-1), is a gut hormone known to play an important role in an in vitro enteric-insular axis. Since GLP-1 immunoreactivity and specific receptors are present in the rat brain, we investigated the biological effects following central GLP-1 administration. GLP-1 was injected directly into the lateral ventricle of brain-cannulated rats, and food and water intake were measured. In cannulated rats with access to food and water, GLP-1 administration for 4 days was associated with significantly reduced food intake as compared to a saline injected control group. Furthermore, GLP-1 produced a strong dose-dependent inhibition of both food and water intake. At doses of 1 and 10 μg of GLP-1, food intake was significantly suppressed to 46 ± 2% (n=9) and 23 ± 6% (n=10) of control animals (n=15). To investigate the effect of GLP-1 on water homeostasis, the urine output of freely feeding rats was measured following intracerebroventricular injection of GLP-1. The urine output was considerably increased by GLP-1 in a dose-dependent manner. At doses of 1 and 10 μg of GLP-1, urine output was increased 4.51 ± 0.88 (n=14) respectively 2.16 ± 0.37 (n=10) times of control (n=34). Measurement of sodium and potassium concentration in the urine samples revealed GLP-1 as a naturetiremic. These significantly effects on both food and water intake and urinary output suggests an important role for GLP-1 as a structurally related peptide as a neurotransmitter in regulation of both digestive behaviour and salt and water homeostasis.

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562.1
CELIAC GANGLIONIC AND VAGAL PROJECTIONS TO THE RAT DUODENUM COMPARED USING DII/DlA DUAL LABELING AND CONFOCAL MICROSCOPY. Z. Cheng and T.L. Powley*. Purdue University, West Lafayette, IN 47907.

The duodenum receives strong autonomic projections. The patterns of these inputs are still incompletely known. To label both branches of the ANS concurrently, male rats were injected bilaterally in the dorsal motor nucleus of the vagus with DIA and then one to two weeks later in the left celiac ganglion with DII, after prior sectioning of the abdominal splanchnics. Five days before sacrifice, the animals were injected i.p. Fluoro-gold injections to label the enteric nervous system. After perfusion, the duodenum was separated into muscularis externa and submucosal sublaminae and processed for confocal microscopy. In contrast to the vagal pattern of dense networks of fibers and varicosities innervating the myenteric ganglia, sympathetic fibers tend to course superficially around the perimeter of the ganglia. These celiac fibers provide fine collaterals within some ganglia but form relatively few varicose contacts. Celiac fibers also form lattices of axons between the longitudinal and circular muscle layers. These lattices are comprised of axons and interstitial cells paralleling the two muscle sheets. Other fibers penetrate into one or the other muscle layers and arborize in varicose endings. These intramuscular effector fibers parallel the muscle fibers of the respective layers. In the submucosa, the sympathetic effector form extensive networks of endings both on blood vessels and on interstitial cell complexes. They also supply varicose endings to submucous ganglia. Single vagal varicosities, as well as rings and baskets of vagal endings, occur in apparent contact with those myenteric neurons which retrogradely label from the celiac ganglia. These fibers have been injected in the ipsilateral nodose ganglion with WGA-HRP or DII. All rats had i.p. Fluoro-gold injections to counterstain enteric neurons and verify the rhizotomy. Axons were perfused and processed with DMB (WGA-HRP) or for confocal microscopy (DII). The entire stomach and oesophagus are also examined. These results may shed light the contribution of these axons in the enteric system. NIH DK27627, NIMH MB10152 & Special Initiative Fellowship to Z. C.

562.2
DIFFERENT VAGAL EFFERENT PROJECTION PATTERNS IN STOMACH, DUODENUM AND CEUM. J.B. Kelly*, M-C. Hoist and T.L. Powley. Purdue University, West Lafayette, IN 47907.

In a recent review of vagal motor fibers in the gastrointestinal tract, we observed that vagal efferents collateralize extensively, providing a dense network of endings within enteric plexuses (cf. Boyd et al., Neurosci. Abst. 19:243, 1993). We present in brief the results of the present study that characterize and compare patterns in three prominent GI organs distinguished both by projections from different motor neuron pools within the dorsal motor nucleus of the vagus and by their specialized functions. Male rats received one or more intraperitoneal injections of PHA-L in the dorsal motor nucleus of the vagus and were perfused 21 days later. Whole mounts of stomach, small intestines, and ceca, as well as frontal sections of the medullas of guinea pigs (56m), were prepared immunohistochemically (Vector ABC Elite Kit). Auranofin blue was used to counterstain both enteric neurons and the medulla. Individual motor fibers were identified as they entered the target organs and then traced and digitized in their entirety (Euretics Trax Imaging System). In the stomach, vagal efferents enter from the lesser curvature and ramify so extensively, both radially and longitudinally, that single myenteric ganglia (and many individual neurons) receive multiple overlapping, convergent inputs. The proximal duodenum is innervated by two distinctive types of vagal fibers which frequently converge on the same ganglion. One is a longitudinally projecting axon, and the other a radially coursing axon. In the cecum, vagal fibers enter from the mesenteric attachment and fan out through the myenteric plexus to form contiguous radial fields without extensive overlap. Fibers in all three regions, however, have large projection fields, but variations in the amount of ramification and ratio of longitudinal to radial orientation produce differences in the amount of convergence observed in the enteric ganglia of the organs. NIH DK27627 and NIMH MB10120.

562.3
TAXONOMY OF VAGAL AFFERENT PROJECTIONS TO THE GASTROINTESTINAL TRACT. F.E. Wang* and T.L. Powley. Purdue University, West Lafayette, IN 47907.

Vagal afferents have critical roles in the control of gastrointestinal function, but their morphologies and innervation patterns have only been characterized in limited areas. To survey these afferents, including a comparison of their (ar) regional morphologies, (b) projection field sizes, and (c) regional densities, male SD rats were given unilateral intramuscular motor rootlet rhizotomies (adapted from Witzel & Williams, J. Aut. Nerv. System, 1991, 38:15-45) and then injected in the ipsilateral nodose ganglion with WGA-HRP or DII. All rats had i.p. Fluoro-gold injections to counterstain enteric neurons and verify the rhizotomy. Animals were perfused and processed with DMB (WGA-HRP) or for confocal microscopy (DII). The entire stomach and oesophagus as well as the duodenum were examined. This regional study of vagal afferents may provide important insights into the role of the vagus in gastrointestinal function. The NIH and NIMH support these studies.

562.4
DISTENSION INDUCED EXPRESSION OF C-FOS IN MYENTERIC PLEXUS OF GUINEA PIG SMALL INTESTINE. R.C. Ritter* and M. Costa. Dept. of VCAPP, Washington State University, Pullman, WA 99164 and Dept. of Human Physiology, School of Medicine, The Flinders University of South Australia, Bedford Park, S.A.

Distension of the small intestine activates enteric reflexes which involve sensory and motor neurons within the myenteric plexus. In order to identify enteric neurons that respond to a distending stimulus, we examined the myenteric plexus for expression of the immediate early gene, c-fos, following distension. Three cm segments of the jejunum or upper ileum were placed in a 37°C Kreb's buffer as open tubes, or closed sacs. Sacs were distended with 0.4 ml Kreb's buffer or left non-distended. Distension resulted widespread expression of fos-like immunoactivity (Fos-li) in nuclei of myenteric neurons. Neuronal nuclei expressing Fos-li were much less numerous in myenteric plexus of open tubes and non-distended sacs. Application of tetrodotoxin (1μM) in the bath and lumen reduced expression of Fos-li following distension. These results indicate that distension leads to increased expression of c-fos in the nuclei of myenteric neurons as a result of increased neuronal activity. *R.C. Ritter was Visiting Professor and Senior International Fellow at Flinders University during the conduct of these experiments.

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R.L. Stephens 0.1 0.13 1376 1376 67.0 3.4 sec depolarization with an amplitude of 5.0 ± 0.4 mV that had a reversal potential potential near 0 mV. The response was associated with a 23.5 ± 1.8 % decrease in input resistance. The magnitude of the depolarizing response was dependent on the duration of CGRP application, and was diminished in a low Na+ bathing solution. During the depolarizing response, neurons had increased excitability, manifested as an increase in numbers of spikes per current pulse, and activation of anodal break action potentials. These results indicate that CGRP, along with substance P, may mediate slow EPSPs in gallbladder ganglia and that its depolarizing action is due predominantly to opening of cation channels. Supported by NS26995.


Chemical stimulation of medullary raphe neurones induces gastric secretion, motility, and cytoprotection and ulcer formation through vagal pathways in urethane-anesthetized rats. The effect of excitation of raphe pallidus (Rpa) neurones on the endocine pancreatic secretion was examined. Methods: Rats (200-350 g) were anesthetized with pentobarbital, a PE-50 cannula was inserted into the left cervical aorta, and a double-lumen cannula was placed into the forestomach for measuring gastric acid secretion. Serum insulin and glucagon levels were tested using Linco RIA kits. Baseline Microinjection of kaolin (0.3 ml/kg) into the Rpa induced a increase in serum insulin levels. Values before and 30, 60 and 90 min after microinjection were 0.34 ± 0.22, 0.54 ± 0.06, 0.60 ± 0.09 and 0.13 ± 0.07 mU/ml. All values after Rpa stimulation were statistically significant from the basal level. In the same rats, stimulated gastric acid secretion was also observed (basal: 2.3 ± 0.55; 30 min after Rpa stimulation: 26.1 ± 8.65 ml/g/hr). Microinjection of a saline solution into the Rpa had no effect. Serum glucagon level (basal: 48.8 ± 4.3 pg/ml) significantly increased only 90 min after the Rpa stimulation (86.6 ± 11.2 pg/ml). The increases of gastric acid secretion and serum insulin levels were completely prevented by bilateral cervical vagotomy whereas the increase in glucagon levels was only slightly but not significantly reduced. Conclusion: Chemical stimulation of Rpa neurones not only gastric function but also endocrine pancreatic secretion through vagal pathways.

SEROTONIN INHIBITS, BUT 5-OH-DPAT STIMULATES, GASTRIC ACID SECRETION THROUGH VAGAL-DEPENDENT MECHANISMS. K.J. Leppard*, G.Goldner and R.J. Stephen*, Dep. of Physiology, The Ohio State University, Columbus, 43210.

Serotonin (5HT) is a neuroendocrine component of the gastrointestinal tract. 5HT receptors have been demonstrated autoradiographically in high density in the rat stomach. However, the physiologic role of 5HT, 5HT receptors in the stomach is not known. Thus, the effects of the selective 5HT1 and 5HT2 agonists, 8-OH-DPAT, on gastric function was measured in isolated rat stomach preparations. In acute, urethane-anesthetized rats with gastric fistula, acid secretion was stimulated by pentagastrin (100 mU/kg iv). After 90 minutes, either 5HT or 8-OH-DPAT (5.5 μmol/kg, i.v.) was given. 5HT inhibited, but 8-OH-DPAT stimulated, gastric acid secretion (mean ± SEM % change: 5HT = 47 ± 11; 8-OH-DPAT = 71 ± 42; p<0.05). Bilateral, cervical vagotomy did not reverse the effect of either 5HT or 8-OH-DPAT on acid secretion. In addition, the enhancement of acid by 8-OH-DPAT was spiperone-sensitive. These data suggest that selective activation of 5HT1 receptors may augment gastric secretory function. Supported by NIH DK 42880.

GASTRIC MUCOSAL EROSIONS PRODUCED BY NMDA INFUSIONS INTO THE LATERAL HYPOTHALAMUS-EFFECTS OF SELECTIVE KNIFE CUTS. C.V. Grilj* and A. Landeira-Fernandez and Stacey Nuccio. Department of Psychology, Univ. of California, Los Angeles 90024.

Bilateral infusions of N-methyl-D-aspartate (NMDA) into the lateral hypothalamus (LH), which interrupt intrinsic neurones, produce gastric erosions in rats (Grilj, Rios-Jimenez & Landeira-Fernandez. Soc. Neurosci. Abst., 19, part 2: 1993, 509). The present study attempted to determine the pathways mediating this effect. Infusion of NMDA into LH evoked gastric erosions in rats. In rats whose LH infusions were followed by bilateral thigh muscle infusions, no gastric erosions were observed. Animals receiving Sham min and infused with NMDA exhibited significantly more gastric erosions than those infused with PBS (p<0.05). Lateral parasagittal LH blocks were performed to determine the contribution of NMDA, whereas anterior coronal LH significantly increased the incidence of erosions produced by NMDA (p<0.005). Posterior coronal LH did not significantly increase the incidence of erosions produced by NMDA. These results suggest that intrinsic LH neurones with gastric function project axons laterally and probably descend through the internal capsule to brainstem mediatory nuclei. The results of the anterior coronal LH suggest that the LH send and/or receives inhibitory projections from neuronal structures (possibly the amygdaloid complex) anterior to the plane of the LH.
562.11 REGULATION OF AUTONOMIC FUNCTIONS: GASTROINTESTINAL

562.12 CONSTITUTIVE AND INDUCIBLE NUCLEAR ONCOPROTEINS IN THE ENTERIC NERVOUS SYSTEM OF THE GUINEA PIG ILEUM. E.J. Parr and K.A. Shakery. Neuroscience Research Group, The University of Calgary, Calgary, AB, Canada T2N 4N1. Constitutive c-Myc and c-Fos are overexpressed in the nuclei of all enteric neurons of the guinea pig ileum. We have demonstrated that they make suitable markers to count enteric neurons in double-labelled preparations as well as providing a method to distinguish neurons from glia.

We have also investigated inducible nuclear oncoproteins in the guinea pig enteric nervous system. Inducible nuclear c-Fos, JunB and c-Jun immunoreactivity was found to be elicited in neurons by in vitro incubation of ileal segments in media containing 50mM K+ or 20mM veratridine as depolarizing stimuli. The neurons expressing activity-related c-Fos antigens included all major immunohistochemically defined subgroups of the submucous plexus. However, in incubated ileal segments, smooth muscle cells and substantial numbers of glia as well as submucous neurons containing VIP-immunoreactivity expressed nuclear c-Fos-immunoreactivity in the absence of a depolarizing stimulus. We conclude that these immediate early genes appear to be regulated by neuronal activity in most or all subgroups of neurons but in vitro isolation of ileal segments is sufficient to induce expression of these genes in many cell types as yet unidentified stimuli. Supported by the Medical Research Council of Canada.

562.13 MUCOSAL EXCITATORY INFLUENCE ON ANATOMICALLY IDENTIFIED NEURONS IN THE MYENTERIC PLEXUS OF THE GUINEA-PIG ILEUM. W.A. Karpe, J.C. Bornstein, J.B. Farnes and H.M. Young. Departments of Physiology and Anatomy & Cell Biology, University of Medicine, Parkville, Victoria, Australia 3052.

To examine the influence of input from the mucosa on 5 neurons of the myenteric plexus, we compared the electrical properties of neurons close to the mucosa with those whose somata lay further away. Opened segments of guinea-pig ileum were superfused with oxygenated physiological saline at 37°C; contractions were maintained by adding 1mM of nicotine. The myenteric ganglion was exposed for half the area of each segment by removal of overlying muscosa, submucosa, and circular smooth muscle. Intact muscosa was circumferentially adjacent to the exposed plexus. Neurons were impaled with conventional micropipettes filled with 1M KC1 and 4mM neurobiotin™ (Vector) and subsequently visualized using Texas red fluorescence. Twenty-three neurons were recorded adjacent (<500 µm) to and 13 far (>1000 µm) from the mucosa. Twelve adjacent neurons, but only one far neuron discharged tonically throughout 500 ms depolarizing current pulses, whereas 11 adjacent and 12 far neurons fired phasically. None of the tonic neurons projected orally; however, 17 of 19 had axonal projections, but 1 far neuron was impaled >500 µm from the mucosa projected orally. The results indicate that input from the mucosa may preferentially enhance the excitability of orally projecting 5 neurons.

562.14 NEURAL CONTROL OF GASTRIC ACID SECRETION: ANATOMICAL DEMONSTRATION OF VAGAL INPUT TO GASTRIN RELEASING PEPTIDE (GRP) CONTAINING ENTERIC NEURONS. H.R. Bartleby* and Q. Lin. Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA 70808.

The prevailing theory of vagal control of gastric acid secretion suggests the presence of at least two stimulatory pathways. One is a direct cholinergic input to the parietal cells, the other involves the release of gastrin from mucosal endocrine cells that is partially driven by GRP containing vagal postganglionic enteric neurons. Additionally, an indirect cholinergic input mediated by somatostatin cells. In order to anatomically and neurochemically characterize this vago-enteric interface, we started to examine the relationship between DA labeled vagal preganglionic terminals and immunocytochemically characterized enteric neurons and their projections. The percentage of GRP-positive myenteric neurons is gradually decreasing from about 40% in the fundus or forestomach, to 25% in the corpus and only about 10% in the pyloric antrum. Dense networks of GRP-positive varicose terminal fibers were found around gastric glands, in myenteric ganglia, and more sparsely within the external smooth muscle layers. In the corpus and antrum, 34% and 35%, respectively, of the GRP-positive neurons receive vagal efferent contacts. Conversely, of all the vagally contacted neurons, 33% and 16% were GRP-positive in the corpus and antrum, respectively. Vagal contacts with enteric neurons of other neurochemical phenotypes and with somatostatin cells, as well as possible contacts of DR-labeled vagal afferent fibers within such neurons, are currently being analyzed. In conclusion, there is a significant number of GRP-positive enteric neurons that receive vagal preganglionic input, supporting a role of GRP in vagally controlled gastrin and gastric acid secretion and possibly other functions. Supported by NIH grant DK74348.


The role of the vagus nerve in the regulation of water and electrolyte transport across the small intestine is well documented. However, there is lack of adequate information on its role in amino acid absorption. This study was thus undertaken to characterize the role of the vagus nerve in jejunal alanine absorption, using selective chemical stimulation or block of various groups of vagal afferents and efferents, and the single-pass perfusion technique. Implication of capsaicin (cp) to the isolated and intact cervical vagi produced a 14% decrease of alanine absorption while similar application to the distal parts of cut vagi produced a 26% inhibition of alanine absorption. Chronic destruction of the cp sensitive vagal afferents resulted in 40% increase of alanine absorption. On the other hand selective activation of preganglionic vagal neurons by microinjection of kainic acid into the dorsal motor nucleus of the vagus produced a 48% increase in jejunal alanine absorption; this increase was abolished by previous treatment of the injected rats by atropine. These results suggest that cp sensitive vagal afferents exert an inhibitory function of jejunal alanine absorption while cholinergic myenteric vagal afferents exert an opposite facilitatory effect. (Supported by grants from the L.N.C.S.R. and O.R.S.B.).


Intracellular recordings were used to examine the actions of putative adenylyl cyclase-activating peptide (PACAP) on morphologically identified neurons in the guinea pig pancreas. In addition, distribution of PACAP immunoreactivity in the pancreas was examined using an antibody that recognizes PACAP-27 and -38. Pancreatic neurons were classified as tonic, phasic, or nonspiking based on their physiological activity. PACAP-38 applied by pressure microinjection in phasic pancreatic neurons. The responses consisted of membrane depolarization in association with increased input resistance and repetitive phasic membrane discharge. Nonspiking neurons became excitable after application of PACAP. Most of the phasic pancreatic neurons that responded to PACAP had filamentous processes that were densely surrounded by VIP-immunoreactive terminals. PACAP immunoreactivity was observed in nerve fibers and in varicose terminals that covered a subset of pancreatic neurons. PACAP immunoreactive pancreatic neurons were not observed; however, they were observed in myenteric ganglia in the adjacent duodenal region. The effects of PACAP and distribution of PACAP immunoreactive nerve fibers on pancreatic neurons suggest that PACAP is a neuromodulator. The possibility that PACAP immunoreactive acon are of enteric origin is being studied. Supported by grants NS27645 and NS01582.
563.1 DETECTION OF INSPIRATORY RESISTIVE LOADS IN HUMANS TRAINED TO BREATHE WITH DIAPHRAGM OR INTERCOSTAL MUSCLES. S.M. Reynolds*, W.R. Revak, and D.T. Prader. Department of Physiology, University of Kentucky Medical Center, Lexington, KY 40536-0084.

We recently recorded central evoked potentials in response to inspiratory occlusion in humans trained to breathe with their diaphragm or intercostal muscles (Neuromuscular, Abst 19/76, 1993). The amplitude of the evoked signal during inspiratory breathing was significantly greater than that for any other condition. One explanation for this could be that the greater amplitude is related to the greater number of persistent afferents in intercostal muscles compared with that of the diaphragm. The objective of the present study was to determine whether differences for the detection threshold of added resistive loads during free breathing (FB), diaphragm breathing (DB), and intercostal breathing (IB) exist. Assuming a correlation between perception of added loads to breathing and evoked potential amplitude exists, we hypothesized that the threshold for detection using intercostal breathing would be lower than that for diaphragm breathing. Respirate was used to monitor abdominal and rib cage movement. Subjects were provided visual feedback by watching the output of each channel on an oscilloscope. Nineteen inspiratory resistive loads (26-3.54 cmH2O/L/sec) were presented to ten subjects for a single breath under three conditions (FB, DB, IB). The detection threshold (mean ± SEM) was 0.53 ± 0.02 cmH2O/L/sec and 0.09 ± 0.09 for IB. There was no statistically significant difference between these means. There appears to be no significant correlation between evoked potential amplitude elicited by inspiratory occlusion and detection threshold for resistive loads across the breathing conditions. (Supported by NIH Grant # PO1 40369)


When supine, abdominal muscles are silent. However, they are active in expiration during an inspiratory threshold load (ETL) or during continuous positive airway pressure (CPAP). We questioned how the sensory inputs of these two loads are integrated in abdominal muscles. Both loads decreased end tidal volume (EELV), and hence, vagal-volume feedback, but CPAP modifies feedback from chest wall receptors as well. EELV was passively increased to 25% and 50% of inspiratory capacity (IC) by rapidly reducing pressure in a full chest cuirass. EELV was actively increased by breathing against a water column. We measured changes in breathing pattern and in expiratory activity in surface EMGs from external oblique (EO) and internal oblique (IO) abdominal muscles during ETLs of 5, 10, and 15 cm H2O on supine subjects (5s). We used a “Respirate” to record EELV changes, and a pneumotach to assess Vt, Tt, and Tc. An increase in ETL evoked deeper, slower breathing, and higher peak expiratory activity in EO and EO, with minimal change in burst duration. Breathing was not altered by inflation to 25% IC. Inflation to 50% IC increased Vt, without changing timing, and in 2 s evoked expiratory activity in I0. Combined ETL and CPAP evoked no additional changes in breathing pattern or burst duration, but did elevate the EO and I0 expiratory peaks more than either load alone. Thus, abdominal muscles during the combined loads had additive effects on the output of the central pattern generator, but not on that of the central rhythm generator.

563.3 METABOLIC AND RESPIRATORY RESPONSES TO MILD EXERCISE IN PATIENTS WITH CFS DL. Cordova, J.C. Pareja, R. Bredon, N.N. Tapp, and B. NClinical. Neurobehavioral Unit, University of Buffalo, Buffalo, NY 14218.

To identify patients with Chronic Fatigue Syndrome (CFS), our lab has been studying the physiological responses that CFS patients exhibit during and after treadmill exercise. Seven medicated CFS patients and seven sex-matched controls screened for their level of fitness were recruited for this study. Subjects walked on a motorized treadmill at 2.5 mph for 4 minutes and then sat for 4 minutes. This pattern repeated 4 times. Physiological variables were collected from an ECG machine and a metabolic breath analyzer. Baseline heart rate, tidal volume, minute volume, respiratory rate, V02 and RQ were not different between groups. There was a significant difference in end-tidal CO2 during the initial resting stage (CFS: 32.6±3.8; controls: 35.3±4.0, mean ± SEM, p<0.01). For the entire test, ANOVA revealed significant group differences for respiratory rate and ventilation, which were higher for CFS patients and end-tidal CO2, which was lower for CFS patients (p<0.05). The decreased end tidal CO2 at rest does not correlate with hyperventilation and so may reflect either a physiological abnormality or pre-test hyperventilation. (Supported by NIH 1AI-23247 & VA Medical Research Funds)


Respiratory related evoked potentials (RREP) were previously recorded with inspiratory occlusion. We hypothesized that a RREP can be recorded using expiratory occlusion. Subjects were scanned while lying in supine, screen the experimentier and apparatus. They respired through a mouthpiece and non-rebreathing valve. The expiratory occlusion was produced using a pressure activated valve with a 2 sec closure time. Early occlusion was interrupted with an occlusion for 30 sec. EEG activity was recorded from F3, F4, C3, C4, T3, T4, P3, P4, T5, T6, C3 and C4 referenced to the linked earlobes. The maximum th of 80 occlusions were separated by a control trial on 80 no-load presentations. The EEG activity was computer averaged and saved on disk. Expiratory occlusion elicited an evoked potential was found in all subjects. An initial positive peak (P1) was the only consistent peak observed in all subjects and was present in electrodes C3, C4, T3, T4, P3, P4, T5, T6, C3 and C4. A negative peak was observed in electrodes F3, F4, T3, T4, P3, P4, T5, T6, C3 and C4. An initial positive peak latency was measured 42 sec. No activity was consistently observed in the frontal electrodes. No peaks were observed with control trials. These results suggest the hypothesis that a RREP can be recorded with an expiratory load. The distribution of the P1 peak is consistent a bilateral sensory cortex origin. The relative latency is similar the P1 peak found with inspiratory occlusion suggesting that this expiratory positive peak is the initial respiratory neuroreceptor mediated activation of the sensory cortex. Supported by NIH/NHLBI grant HL45782.


In man, some putative brain sites mediating responses to respiratory loading are unknown. To examine this issue, we employed a 1.5 Tesla MR scanner, and used SPGR pulse sequences (TR: 72 msec; TE: 45 msec; flip angle: 30; FOV: 26 cm; slice thickness: 5 mm; 128 x 256 x 1 NEX). Two-channel magnetic and axial images across the rostral pons were acquired in 8 healthy volunteers during two-way valve unloaded breathing, and while applying a 30 cm H2O resistor to the inspiratory port. Digital image subtraction and ROI analysis revealed significant increases in image intensity in discrete regions of the dorsal medulla, pons, and cerebellum, as well as bilaterally, in the inferior colliculi, putamen, and internal capsule. With continuous loading, signal increases progressively diminished over time. Upon cessation of stimulation, signal gradually returned to baseline. We conclude that inspiratory loading elicits consistent regional activation of discrete brain locations. We speculate that temporal changes in signal intensity may indicate respiratory afterdischarge and habituation phenomena. (Supported by HL-24218, HD-22695 & Parker B. Francis Foundation)


Functional MRI provides a noninvasive tool to assess spatial and temporal characteristics of neural responses to respiratory loading. We attempted to identify human brain regions which may undergo the ventilatory response to increased respiratory resistance. To study this issue, we employed a 1.5 Tesla MR scanner, and used SPGR pulse sequences (TR: 72 msec; TE: 45 msec; flip angle: 30; FOV: 26 cm; slice thickness: 5 mm; 128 x 256 x 1 NEX). Mid-sagittal and axial images across the rostral pons were acquired in 5 healthy volunteers during two-way valve unloaded breathing, and while applying a 30 cm H2O port. Digital image subtraction and ROI analysis revealed significant increases in image intensity in discrete regions of dorsal medulla, and the ventral and dorsal pons. With continuous loading, increases in signal progressively diminished over time. Upon cessation of stimulation, ROI signals immediately returned to baseline. We conclude that expiratory loading elicits consistent regional activation of discrete brain locations which differ from those which uniformly activate during inspiratory loading. We speculate that temporal changes in signal intensity may indicate either a switch to oxidative neural metabolism or habituation phenomena. (Supported by HL-24218, HD-22695 & Parker B. Francis Foundation)
563.7

NUCLEUS AMBIGUUS AND THE VENTRAL RESPIRATORY GROUP: A CORRELATIVE ANATOMICAL STUDY OF THE RAT AND HUMAN VENTROLATERAL MEDULLA OBLONGATA. H. H. Eickelberg and D. A. Hopkins, Department of Anatomy and Neurobiology, Dalhousie University, Halifax N.S., Canada, B3H 4H7.

The nucleus ambiguous (NA) and ventral respiratory group (VRG) in the ventrolateral medulla of the human brainstem were characterized by an anatomical comparison with homologous neuron populations in the rat ventrolateral medulla. To identify these neuron populations, we performed parallel immunohistochemical labeling studies on rat and human brainstem tissue using antibodies to choline acetyltransferase (ChAT) to label NA motor neurons, and parvalbumin (PV) to label presumptive bulbar VRG neurons (Cox & Halliday, Neurosci. Lett. 160:101-105, 1995). Anatomical subdivisions of NA and VRG were identified in the rat in transverse and sagittal sections of ChAT-immunoreacted tissue. ChAT-immunoreactive neurons in the ventrolateral medulla of the human brainstem were distributed in locations and patterns that were comparable to those in the rat NA. In particular, the compact division of NA formed a distinct dorsal column in both species. By combining PV immunohistochemistry with retrograde labeling after Fluoro-Gold injections into the C4 spinal cord, we have shown that bulbar VRG neurons are PV-immunoreactive in rats. Our comparison of the location and distribution of PV-immunoreactive neurons in the ventrolateral medulla of the human and rat brain suggests the presence of a homologous of the rat bulbar VRG neuronal population.

Supported by MRC grant M-2112 (HDL) and MT7356 (DAH) and a grant from The Scottish Rite Charitable Foundation (DAH).

SOMATIC AND VISCERAL AFFERENTS: SOMATOSENSORY

564.1


Unlike heat pain, the peripheral neural mechanisms underlying sensation of cold pain are poorly understood. Although many studies have examined responses of cutaneous nociceptors to noxious heat stimulation, responses have often not been well described, and responses to cold stimuli have not been well characterized. We have examined responses of cutaneous nociceptors to noxious cold stimuli. Single fiber recordings were made from the suprachinnae nerves in anesthetized monkeys. Nociceptors were located by nailing 18-20 cm, the skin and receptive fields mapped using von Frey filaments. Conduction velocity, mechanical threshold (von Frey), and response to thermal stimuli were assessed. Heat stimuli of 59.1-7°C (5 sec duration) and cold stimuli of 30 to -14°C (5 or 10 sec duration) were applied from a base temperature of 32°C and delivered in ascending or descending order. Recordings were made from 9 A and 6 C-fiber nociceptors. Mechanical thresholds for A and C-fibers ranged from 3.5-8.43 m/s and from 0.1-14 m/s, respectively. Under normal conditions only one A-fiber was excited by noxious heat. In contrast, all were excited by noxious cold stimuli with a mean response threshold of 4.2°C (range: 2.8 to 12.5°C). 7 of 9 A-fibers had a response threshold below 0°C. All C-fiber nociceptors were excited by both noxious heat and cold stimuli. The mean response threshold to cold was 15.3°C and thresholds ranged from 30°C to -2°C. Unlike A-fibers, 5 of 6 C-fibers exhibited response thresholds > 0°C. Responses of both A- and C-fibers typically increased monotonically as stimulus temperature decreased. It is concluded that: 1) A and C "mechanonociceptors" are excited by noxious cold and 2) C nociceptors contribute to cold pain at temperatures above 0°C, while both A and C nociceptors contribute to pain sensation evoked by temperatures below 0°C. Supported by NS31233 and NS29567.

564.2

THERMOSENSITIVITY OF CULTURED TRIGEMINAL NEURONS. T.K. Baumann and M.E. Martenson. Division of Neurosurgery and Department of Pharmacology, Oregon Health Sciences University, Portland, OR 97201

Little is known about the membrane mechanisms involved in thermal transduction between the sensory endings in the trigeminal ganglion. To examine the thermal response properties of cultured somatosensory sensory neurons, we have built a miniature heat stimulus system (Baumann et al., Biophys. J. 66: 442, 1994) which can stimulate individual cells. Whole-cell, patch-clamp recording techniques were used to study the thermosensitivity of cultured adult rat, rabbit, and cat trigeminal ganglion neurons. Triangular or rectangular heat stimuli (up to 20°C in amplitude, 3 to 10 s in duration) were applied every 10 to 30 s from a baseline temperature of 25 to 29°C. Responses were non-uniform, but largely reproducible. A proportion of neurons responded to heating with a depolarization and action potential discharge. Others showed the opposite behavior; they were inhibited by heating and discharged action potentials upon heat removal. Some neurons showed no overt response to changes in temperature. Following exposure to high temperatures, some neurons developed spontaneous action potential discharge, some became inexcitable. We conclude that cultured trigeminal ganglion neurons show features of thermoreceptors and heat nociceptors and may provide a useful model for studying thermal transduction mechanisms. (Supported by NSF grant IBN 92-11545.)

564.3


To date, there has been no quantitative, systematic study of the electrophysiology of regenerating cold receptors. This study, therefore, examines the changes in cold-activated neural activity following a circular wound (3 mm dia, 200 um deep) on the surface of the rabbit cornea. This is a well studied wounding model, in which neural regeneration has been anatomically quantified. Extracellular recordings were obtained from a total of 90 single cold fibers, at 1, 3, 10, 20, or 30 days following wounding. Conduction velocities for regenerating cold-fibers were similar to those recorded from normal fibers, 0.6-2.7 m/s, and 0.6-2.2 m/s, respectively. The adapting temperature was 35°C in all experiments. Thermal sensitivity for each fiber was determined using a series of temperature steps, 0.2°C ranging from 35 to 34°C, and 2°C steps ranging from 35 to 24°C. The rate of temperature change (dT/dt) was varied from 0.2 to 1°C/sec for ramps. For sinusoids the frequency ranged from 0.07 to 0.005 Hz at a median temperature of 35°C with a +/-5°C change in amplitude. Action potential frequency (AP) was measured over the magnitude (ΔT, 0 to -9°C) and rate (dT/dt, 2 to 1°C/sec) of temperature change. The action potential acceleration (dAP/dt) and rate of change of adaptation were proportional to dT/dt and independent of ΔT. Sinusoidal stimulation showed that AP always peaked prior to reaching maximal ΔT, indicating that the thermal transducer mechanism does not depend on ΔT. Conversely, and more logically, AP and dAP/dt always peaked just following maximal dT/dt, although the exact phase of the response varied. It is hypothesized that AP is always peaked prior to reaching maximal ΔT, indicating that the thermal transducer mechanism does not depend on ΔT. Convectively, and more logically, AP and dAP/dt always peaked just following maximal dT/dt, indicating that the thermal transducer mechanism does not depend on ΔT.
SOMATIC AND VISCERAL AFFERENTS: THERMORECEPTION


For these experiments, two thermal stimulating probes, 0.5 mm diameter, were employed. Each probe was calibrated so that a temperature change of one degree would correspond to a haptic sensation of the test subject. The probes were then used to determine the haptic localization of thermal stimuli in the dermatomes of the arm and hand. The results of these experiments indicate that the haptic localization of thermal stimuli is not determined by the size of the dermatome, but rather by the size of the region of the body that is supplied by the nerve fibers that are activated by the thermal stimuli. These findings suggest that the haptic localization of thermal stimuli is determined by the size of the region of the body that is supplied by the nerve fibers that are activated by the thermal stimuli.

564.6 TONGUE ADAPTATION TEMPERATURE INFLUENCES THE DYNAMIC AND STATIC RESPONSES OF LINGUAL NERVE FIBERS TO THERMAL STIMULATION. Robert F. Lundy Jr. and Robert J. Conners. The Florida State University, Tallahassee, FL.

The tongue is a highly sensitive organ that is capable of detecting a wide range of temperatures. The aim of this study was to investigate the effects of temperature adaptation on the dynamic and static responses of lingual nerve fibers. The results of this study suggest that temperature adaptation has a significant influence on the dynamic and static responses of lingual nerve fibers. These findings suggest that temperature adaptation is an important factor in the detection of thermal stimuli by the tongue.

ANATOMY AND PHYSIOLOGY OF MECHANORECEPTION


In this study, we used a combination of retrograde HRP and intracellular biotinimide to investigate the synaptic contacts between jaw-muscle spindle afferents and trigeminal motoral thalamic afferents. The results of this study suggest that the jaw-muscle spindle afferents make synaptic contacts with the trigeminal motoral thalamic afferents. These findings suggest that the jaw-muscle spindle afferents play an important role in the control of jaw-muscle movements.


In this study, we developed a circuit model for whisker receptive fields in the nucleus principalis. The results of this study suggest that the circuit model for whisker receptive fields in the nucleus principalis is a complex network of neuronal connections that is responsible for the generation of whisker receptive fields.


In this study, we used quantitative analyses to investigate the distribution of whisker and non-whisker primary afferents in the rat. The results of this study suggest that the distribution of whisker and non-whisker primary afferents is highly organized and is responsible for the generation of whisker receptive fields.


In this study, we investigated the possible inputs of GABA-immunoreactive neurons in the cuneate nucleus of the rat. The results of this study suggest that GABA-immunoreactive neurons in the cuneate nucleus receive inputs from a variety of brain regions, including the thalamus, cortex, and brainstem. These findings suggest that GABA-immunoreactive neurons in the cuneate nucleus play an important role in the regulation of pain and other sensory functions.
565.5 MECHANISTIC MODELS FOR TACTILE NEURAL CODING OF SHAPE. *K.A. Zvonarik* and K. Dandekar. Dept of Mechanical Engineering and Research Lab of Electrics, MIT, Cambridge, MA 02139.

The relationship between the biomechanics of primate fingertip and the responses of slowly adapting mechanoreceptors (SA-I) in the tactile sensation of object shape was studied. Finite element analysis was performed on a progressively realistic series of mechanistic models of the primate fingertip under a variety of shape stimuli. The shape of the models was related to a circular cylinder of about the same diameter as a typical primate fingertip to realistic 3-dimensional stimuli. The latter were generated from video images of accurate epoxy copies of monkey and human fingertips at various known orientations, obtained by using a videomicroscopy system. The internal geometry consisted of rigid bone enclosed by a homogenous or anisotropic material. The stresses, strains and deformations calculated from the models correlated with data from both biomechanical experiments on finger deformations and psychophysical experiments on the responses of SA-I.

In all models, the strain energy density and the maximum compressive strain were the leading contenders to be the relevant stimulus for SA-I. Because the models took into account the curvature and the stiffness of the primate finger, the predictions of SA-I population responses to rectangular gratings indented into the fingertip were more realistic than the predictions from previously proposed flat, semi-infinite models (Phillips and Johnson, J. Neurophysiol. 46(4), 1981). Although these population responses were radically different from the previous predictions, the spatial response profiles matched the neural data as well as before. In addition, the new models predicted that the spatial response profile of a receptor was strongly dependent on its location with respect to the center of contact region on the fingertip. Analysis of the nonlinear mechanics of contact between the models and cylindrical indents of various radii demonstrated good correlation between strain measurements and the relative types and corresponding neural response. (Supported by NIH Grant DC00625 and ONR URI Grant N00014-92-2-1814)

565.7 A VARIABLE THRESHOLD MECHANORECEPTOR MODEL. *P.J. Lock* and K.O. Johnson. 1Dept. of Elect. and Comp. Eng., Worcester Polytechnic Institute, Worcester, MA 01609 and 2Kriger Mind/Brain Inst. and Dept of Neurosciences, Johns Hopkins University, Baltimore, MD 21205.

The responses of primary mechanoreceptive (SA, RA and PC) afferents in macaque monkeys were studied using a sinusoidal vibratory stimulus and a two-pulse condition-test paradigm (CT). The CT experiments consisted of presenting either a mechanical or electrical conditioning pulse followed by a mechanical test pulse. For sub-threshold CT and CT delays 2msec and greater the mechanical test pulse amplitude required to elicit an action potential was essentially invariant - a result that would occur if there was no residual membrane depolarization. Above-threshold conditioning pulse required a significantly increased test pulse amplitude to generate an action potential. Supra-threshold CT experiments showed that threshold recovery following an action potential is slowly fit by a two-exponential decay function with time constants of 4ms and 15-40ms. These results suggest that the previous mechanoreceptive model [J. Physiol. 292:43-64, 1982] was flawed since these authors had proposed that fibers were not immediately fully repolarized following an action potential and that there is a substantial residual membrane depolarization following sub-threshold stimuli. A revised mechanoreceptive model in which threshold recovery was modeled by an exponential decay resulted in significant improvements in fits to the responses of mechanoreceptive afferents to sinusoidal frequency stimuli. In addition to effectively modeling the statistical regularity of the impulse trains, the variable threshold model also properly predicts the initial increase followed by a subsequent decrease in impulse rate with increasing stimulus frequency. Finally, the model predicts that the neural responses are only slightly affected by noise bandwidth or absolute refractory period and are moderately sensitive to the shape of the generator potential.


Impulse activity in axons generates after-effects on membrane excitability that can alter the conduction velocity of subsequently conducted impulses. We used compound stimulus patterns to assess two activity-dependent changes in conduction latency of functionally identified rat cutaneous afferents from the posterior tibial branch of the sciatic nerve. We measured two different parameters of activity dependence: supralinear and sublinear, a decrease or increase in conduction latency following conditioning with a single preceding impulse; depression, an increase in conduction latency during tetani; and recovery, the time course of the baseline conduction latency as measured before and after stimulation. We will present data on the effects of tetanic stimulation on the conduction latency of rat cutaneous afferents. We will discuss how various factors influence this variable and how this study relates to the function of these peripheral afferents. We will also discuss how various factors influence this variable and how this study relates to the function of these peripheral afferents.

Anoxia of Pacinian corpuscles (PC) [10:3] and rapid adapting mechanoreceptors (RA; n=6) of the glabrous skin were examined for activity-dependent changes in conduction latency using a two-pulse stimulus protocol. Following 5 msec isolation RA exhibited sublinearity, i.e., the second of two impulses initiated within 5 msec of another conducted less rapidly than the first. By contrast, only one PC exhibited sublinearity. PC and RA latency changes were directly related to the intensity of the stimulus. The results were consistent with the concept that the axon size of the sensory neuron is a major determinant of the mean normalized difference between the first and second impulse latency: in all three PCs the latency of the second impulse of the burst was normal, while in six of seven RA the latency was subnormal (p<0.05). Our data suggest that the function of mechanoreceptors in the glabrous skin may extend into their axon's conduction properties.

(NIH UHPS GM 35647)

565.6 RESPONSES OF CUTANEOUS MECHANORECEPTOR AFFERENTS TO STEP AND SINUSOIDAL SKIN DISPLACEMENTS. *J.C. Brown* and J. Truog. 1Instute for Sensory Research and 2Department of Bioengineering and Neuroscience, Syracuse University, Syracuse, NY 13244-5290

Neural activity was recorded from 100 mechanoreceptors innervating the glabrous skin of the cat hind-paw. Action potentials from individual nerve fibers of the medial plantar nerve were monitored in response to sinusoidal displacement at a frequency from 1 to 500 Hz and in amplitude from threshold to 250 μm. The same fibers were also characterized in response to step indentations (2 m/sec rise time) to intensities of 100 mm Hz. The responses showed periodicity of firing for the majority of fibers studied. Interval histograms demonstrated that the periodicty ranged from 3 to 35 ms depending upon the fiber type. The PC and RA fibers tended to have shorter interspike intervals in response to the SA fibers. Supported by NINCDS RO1-DC00380.

565.8 ADAPTATION OF MECHANORECEPTIVE AFFERENTS TO CONTINUOUS SINUSOIDAL VIBRATION. *Y.L. Leissa, S.S. Blain* and K.O. Johnson. 3Kriger Mind/Brain Inst. and 4Dept of Neurosciences, Johns Hopkins University, Baltimore, MD 21205.

The effects of stimulus duration, amplitude, and frequency on the adapting sinusoidal stimulus on thresholds of rapidly adapting (RA), slowly adapting (SA) and Pacinian (PC) mechanoreceptive afferents were studied. Recordings were made from medial and ulnar nerves of 3 anesthetized monkeys while vibration was delivered via a 1 mm diameter probe glued to the skin. The sinusoidal stimulus consisted of an adaptation period containing the adapting vibratory stimulus and a recovery period in which no adaptive stimulus was applied. During both periods, a sinusoidal test stimulus was presented once every 4 sec to track changes in absolute and entrainment thresholds. This testing rate was determined to have minimal effects on thresholds. The amplitude of the test stimulus was systematically varied between tests to make sure that the maximum stimulus was always applied. Adapting and test stimulus frequencies of 10, 30, and 60 Hz were used for SAs; 30, 60, and 100 Hz for RAs; and 60, 100, and 300 Hz for a total of 9 combinations of test and adapting frequencies for each afferent type. Amplitudes of the adapting stimulus ranged from 2 to 30 times absolute threshold and were applied for durations of 1, 2, 4 or 8 min. All afferents (6 SAs, 8 RAs, and 5 PC's) exhibit increases in thresholds with increases in either the adapting stimulus amplitude or frequency: there is about a 1 to 10 elevation in threshold over the range of frequencies and amplitudes studied. SA and RA thresholds increase and recover rapidly (t=15-30 sec) during both the adapting and recovery periods with stimulus durations greater than 1 min having no effect on the time constants nor on the level of adaptation. PC afferents adapt slowly (~5x) with the thresholds of some PCs still increasing after 8 min of stimulation. The PC's exhibit variable recovery time constants (30 sec to 6 min) and often have a small residual elevation in thresholds 50 min after the adapting stimulus was discontinued.

565.10 TACTILE SIGNALS GENERATED BY A TOOL USED TO DISCRIMINATE THE SOFTNESS OF OBJECTS. *J.E. LeMotee*, J. Zhang and J. Van. Department of Anesthesiology, Yale University School of Medicine, 335 Cedar Street, New Haven, CT 06510.

Subjects were 90-100% correct in ranking the softness of 9 rubber disks that differed in compliance (1.8 to 100 mm elevation) by indenting each specimen with the fingerpad, or by tapping each with a stylus held between two fingers. In order to study the role of tactile signals, the back of a finger was rigidly mounted to a lever and one rigidly mounted to another. The subject was able to discriminate a soft specimen from a hard one (p<0.05). For soft (≥7.7) specimens and a "medium soft" standard of 4.0 mm/g-wt when the velocity of tapping was held approximately constant. The perception of softness felt "natural" as a tap without percussion. This work is a previously recorded force trace from a specimen was "played back" to the operator. The subjects were unable to discern whether a "live" or a "played- back" trace was delivered. It was found that perceived softness depended primarily on the slope of the initial segment of the rising phase of the force trace which, for hard specimens, changed little with moderate variations in tapping force and velocity in comparison with that for softer specimens. The perceived softness of softer specimens decreased with increasing tap velocity suggesting that, for these specimens, passive touch without independent information about velocity and force was not sufficient for perceptual judgments of softness. It is hypothesized that the greater the initial force rate, the more likely that rapidly-adapting as opposed to slowly-adapting mechanoreceptors will be engaged and thus contribute to the perceived softness of objects. (Supported by ONR URI Grant 00019-92-J-1814)
565.11
INTERLAMELLAR FLUID DYNAMICS OF THE PACINIAN CORPUSCLE (PC). B. Pietrak* and S. J. Balogunski, Institute for Sensory Research and Biomechanics and Neurosience, Syracuse University, Syracuse, NY 13244.

Receptor potentials recorded from isolated PCs in response to sinusoidal vibrations have been shown to have non-linear asymmetrical input-output functions and U-shaped frequency characteristics. The PC's accessory capsule was studied both experimentially and theoretically to determine its mechanical contribution to the observed non-linear receptor potential. During compression of the accessory capsule, a shear stress is created and inter-lamellar fluid flows with velocity components orthogonal to the direction of displacement. The viscosity of the fluid imparts a resistance to the shear stress which arises as a force parallel to the compression. Inter-lamellar fluid is considered to be incompressible and, at the velocities considered herein, to have a resistive force proportional to its velocity. Measurements of reactive force in response to sinusoidal displacements have shown that the reactive force is linearly proportional with increasing frequency and amplitude. Furthermore, the reactive force can be described by a first-order linear differential equation of the form \( \frac{dx(t)}{dt} = k + c \cdot dx(t) \). A series of first-order linear differential equations was used to compute the inter-lamellar fluid velocities and lamellar displacements for a range of frequencies, stimulus amplitudes, and probe sizes. Computational simulations of the inter-lamellar fluid flow in response to sinusoidal displacement of the surface of the capsule shows that the multi-layered structure linearizes the inter-lamellar fluid velocity, thus linearizing the inter-lamellar compressive force. Video analysis of PCs subjected to a static stress shows that the stress load is carried by the outermost lamellae. Indeed, it is only during a highly time-dependent displacement of the surface (frequencies above 40 Hz) that a strain is observed below the outermost lamellae. Video analysis and computational simulations also show that the multi-layered capsule acts as a mechanical high-pass filter. Furthermore, the compressive strain and reactive forces increase with stimulus frequency. Thus, the non-linear asymmetric input-output function and the U-shaped frequency response cannot be mechanical in origin and must arise from intrinsic neural properties of the neurite. Work supported by NSF, BNS-911564.

565.13
A SECOND SYSTEM FOR INNOCUOUS MECHANORECEPTION IN THE HUMAN SKIN. H. Olejnik, N. Rekulu, J. Wessberg and Å. B. Vallbo, (SPON: European Neuroscience Association), Department of Physiology, Göteborg University, Medicinaregatan 11, S-413 90 Göteborg, Sweden.

In various mammals many unmyelinated (C) afferents respond to light touch. In man on the other hand, C-fibers with sensory functions have been identified almost exclusively with the senses of pain and temperature. For that reason it has been suggested that an older and slowly conducting system for innocuous mechanoreception has faded away during the evolution. Using the microneurography technique we have now found low threshold mechanoreceptors with conduction velocities within the unmyelinated region to be frequent in the hairy skin of the human forearm. The impulse rate associated with clearly innocuous mechanical stimulation amounted to 100 imp/s, leaving no doubt that light touch was the adequate stimulus. The thresholds to v Frey hairs ranged between 0.01 and 8 g with a median value of 0.25 g. The conduction velocity assessed using electrical or mechanical stimulation ranged between 0.7 and 1.0 m/s. For some units the receptive fields were explored in detail using a scanning method. The fields were found to be fairly large (10 mm x 5 mm) and composed of multiple areas of different sensitivity. Our study showed that low threshold mechanoreceptors are common in the hairy skin of the human forearm. It suggests that the human body is provided with a widespread system for innocuous mechanoreception subserved by C-fibers.

565.14
A NOVEL METHOD FOR SELECTIVE ELECTRICAL STIMULATION OF SMALL DIAMETER NERVE FIBERS. J.C. Petruska* and R.D. Johnson, Departments of Physiological Sciences and Neuroscience, University of Florida, Gainesville, FL 32610.

There is a good deal of evidence that the small diameter nerve fibers in peripheral nerves, the thinly myelinated A-delta fibers and unmyelinated C fibers, display a high degree of central and peripheral plasticity in response to injury to the nervous system. The electrophysiological investigation of specific spinal cord input from these fibers is difficult because standard electrical stimuli recruit large myelinated fibers before small fibers. We have designed the following method for selectively stimulating small fibers.

In urethane-anesthetized rats, the caudal cutaneous sural and sciatic nerves were surgically isolated. A lumbar laminectomy was performed to isolate dorsal root filaments for recording. A bipolar Ag/AgCl electrode was placed around the sural nerve for stimulation of various fiber groups. Specially fabricated Ag/AgCl ring electrodes producing differential current densities were placed around the sciatic nerve. Using an arbitrary-waveform constant-current stimulus isolator and a modified triangular stimulus waveform, an anodal polarization block of sural-activated large A fibers was achieved at the sciatic site, allowing only the smallest A-delta and/or C fibers to conduct through to the dorsal root filaments.

This method could be used to study the input from small fibers into the spinal cord and/or the peripheral target without tissue damage by large myelinated fibers. Supported by NS 27511.

565.15
INHERENT FIRING PATTERNS OF MUSCLE AFFERENT AXON TERMINALS ARE GRADUALLY LOST IN CHRONIC NEUROMAS. J.B. Munro* and R.D. Johnson, Dept. of Physiological Sciences and Neuroscience, University of Florida, Gainesville, FL 32610.

We have previously shown that following transection and cross-regeneration of cat medial gastrocnemius (MG) muscle nerve into the sural nerve skin territory, large diameter muscle afferent fibers retained their stretch-sensitive and slowly-adapting firing patterns despite the presence of a foreign target devoid of muscle receptors. We have also shown that in short-term 6-day neuromas, even the sprouts of axonotomized muscle afferents retained these native firing patterns. Since these characteristics were evident for at least 2 years in skin, we designed the present study to determine if these firing patterns could be obtained from muscle afferent sprouts in long-term neuromas. In adult cats, the MG nerve was transected at the mid-thigh level and sutured into a blind-ending Gore-Tex sleeve. Following a recovery period of 3-16 months, the mechanosensitivity of sprouted afferents was tested by recording from both root and filament while mechanically stimulating the neuroma. Compared to afferents from 6-day neuromas, those from long-term neuromas were significantly more difficult to mechanically activate and showed a progressive decrease in conduction velocity, stretch-sensitivity, and slow-adaptation properties. We suggest that these of the physiological properties may be dependent on trophic factors present in peripheral targets such as muscle and skin absent in chronic neuromas. Supported by NS 15913 and NS 27511.
SOMATOSENSORY and THALAMOCORTICAL RELATIONSHIPS III

III.1

SHORT-TERM CHANGES IN EFFICACY OF CORTICOSEPTAL EPSPs FROM RACCOON PRIMARY SOMATOSENSORY CORTEX. P. Zorzin and P. Ijzenga. MRC Group in Sensory-Motor Physiology, Dept. of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Neurons in the cortical representation of glabrous skin of raccoon digits receive inputs from "off-focus" digits by direct thalamocortical connections. Weaker and less common inputs from "off-focus" digits are probably relayed by a corticopetal pathway from the heterogeneous zone a few mm rostral to the glabrous zone. "Off-focus" inputs quickly become more effective during short-term cortical plasticity, a change that could contribute to the formation of corticocortical synapses.

Our purpose was to test for short-term plasticity in the corticopetal pathway from the heterogeneous zone to the glabrous zone. Neurons were recorded intracellularly in the glabrous sheet of glabrous skin in the raccoon primary somatosensory cortex of raccoons anesthetized with nembutal. Short latency corticocortical EPSPs were masked following electrical stimulation (5 Hz) of the heterogeneous zone. Cortico-cortical synaptic responses were paired with simultaneous depolarizing current steps (up to 3 nA, 100 ms duration) injected intracellularly. Pairs of EPSPs with depolarizing current steps (50 pairings at 5 Hz) caused enhancement of corticocortical EPSPs lasting beyond the period of pairing. Enhanced EPSPs that could be relayed "off-focus" somatosensory inputs can be enhanced by over by depolarization of the target neuron by another input. Remaining questions are whether somatosensory EPSPs are also enhanced after cortico-cortical EPSPs are paired with current steps, and whether any such effects on somatosensory responses are restricted to "off-focus" inputs.

Supported by the Medical Research Council of Canada.

III.6.2


Clustered intrinsic connections in the cat somatosensory cortex (SSC) develop from an initially diffuse network. Although the shift from reverse homology to intermittent clusters can be observed visually, the transition is not easily defined. As a result, we found it important to quantify the developmental sequential identity of the changes that occur in intrinsic cortical development. To do this, confocal images were obtained from cat somatosensory cortex (SSC) of light green and gli-labeled kitten SSC at various postnatal ages. Several statistical tests were conducted in order to analyze the clustering of retrogradely labeled neurons and the distribution of tangential fibers emanating from the injection site. For some tests, individual sections were drawn to indicate labeled neurons. We analyzed neuronal neuronal clustering using spatial point process analysis. The null hypothesis, that the structure of labeled neurons at each age, did not differ from CSR (complete spatial randomness). Quad contours showed a change from a random distribution on PND3 (p<.001) to a clustered one on PND6 (p<.001). A refined nearest neighbor analysis confirmed conversion from a random distribution on PND3 (p<.43) to a clustered distribution on PND6 (p<.01). Other tests (disposition indices, density recovery profiles, etc.) verified the transformation of a diffuse organization of neurons to a clustered one. Coronal sections on PND 15 confirmed a pathway distribution of intrinsic connections resembling the adult distribution. The numbers of labeled neurons were significantly higher in clusters than in the intercluster zones (p<.001) on PND6. Profile analysis of the barrel hollo revealed a significant periodicity (center-to-center ~ 750um) on PND6. We demonstrate that the study of local circuit development of the cortical cortex is amenable to formal statistical analyses. Support: PHS NS24014.

III.6.3

SEQUENCE OF EVENTS IN THE DEVELOPMENT OF LAMINATION, INTRINSIC AND EXTRINSIC CONNECTIONS IN FERRET SOMATOSENSORY CORTEX. S.L. Julian*, R.V. Sonty, S. Palmer, P. Asimowicz, Depts. of Anatomy & Neuroscience, USUHS, Bethesda, MD.

Despite the relative immaturity of ferret neural system at birth, we previously demonstrated that axons of thalamic neurons reach the somatosensory cortex by PND 1, and are organized into distinct clusters by PND 5. Horizontal connections, in contrast, do not display a clustered organization until PND 28. To more precisely define the development of intrinsic and extrinsic connections in relation to the maturation of the cerebellum, we used slices of ferret pups aged PND 1-6. After injection of fluorescein-labeled dextran the slices were maintained in an oxygenated chamber to allow for transport of the tracer. On PND 1-3, injections into the deep cerebellar nuclei or, in subcortical white matter (WM), led to a slender radial column of labeled cells that extended from the upper portion of the cortical plate to the base of the ventricular zone. The labeled radial column contained both radial glia and neurons and their processes were evident within the soma. The cells within the column did not extend axons into the cortex, although horizontally-running fibers were seen beneath layer 6. In contrast, injections in the superficial cortical plate led to labeled neurons that extended their processes tangentially. Comparison with the locations of cells labeled with BRDU and born from 4E2-E38, indicate that neurons destined to occupy all cortical layers are present in the cortex by PND 3. At PND 1, injections into the white matter labeled thalamicocortical axons that terminated in crude clusters within the soma. With increasing age, the deeper injections lost their radial character and by PND 14 many axons extend into all cortical layers. After injections into more superficial cortical sites, a continuous band of labeled cells can be seen in layers 2-3. By PND 28, injections into the upper layers result in discrete clusters of labeled cells in layers 2-3. Supported by NS24014.

III.6.5

FACTORS THAT INFLUENCE NEURONAL RESPONSE VARIABILITY IN SOMATOSENSORY CORTEX: THE ROLE OF NMDA RECEPITORS. D. Prince, B. Whitham, M. Tommerdahl, O. Fayou, Departments of Biomedical Engineering and Physiology, University of North Carolina, Chapel Hill, NC 27599.

Potentials evoked in rat somatosensory cortical slices by single-stimulation of the white matter were examined (i) to determine the effects of stimulus frequency and intensity on intracellular response variability (hereafter referred to as response variability in SI neurons, and (ii) to evaluate the prediction that response variability is an indicator of the degree of cortical NMDA receptor activation (Lee and Whitham, 1995). Evoked potentials (EP's) were recorded for 50 to 100 consecutive stimulus presentations at a given stimulus frequency and intensity (frequencies between 0.2 - 5 Hz and intensities between 1.0 - 3.5 times the minimum intensity for evoking a response). NMDA EP's were recorded for each trial was determined by integrating the EP over the duration of the post-synaptic component; response variability was calculated as the coefficient of variation of the EP magnitude from the EPs recorded for each trial. To ascertain the contribution of the NMDA receptor population to response variability, some experiments were performed following application of ketamine, a selective NMDA receptor antagonist, and penicillin, which was found between response variability and stimulus intensity. As intensity was increased, variability decreased linearly, and the slope increased over the range 0.2 to 5 Hz. The relationship between stimulus intensity and variability of effects of stimulus frequency on this relationship was observed more clearly at more than superficial sites in SI cortex (those containing the highest density of NMDA receptors). (Here, variability decreased the dependency of response variability on stimulus intensity in a dose-dependent fashion. This work was supported in part by NIH RO1 MH48464 and the Whitaker Foundation.

III.6.6

NMDA RECEPTOR EXPRESSION IN THE RAT TRIGEMINAL PATHWAY TO BARRELL FIELD CORTEX DURING DEVELOPMENT. V. Remley and P. F. Brown. Institute for Developmental Neuroscience, Kennedy Center, Vanderbilt University, Nashville, TN 37203.

Polycyclic antibodies against the C-terminal and N-terminal domains of the NMDAR1 subunit were produced to examine changes in NMDA receptor expression during normal postnatal development of the trigeminal pathway to rat barrel field cortex. At P10 there is already substantial expression of NMDAR1 subunit in the trigeminal nucleus (V1) containing intra- and interlaminal thalamic nuclei, and in barrel field cortex. From birth to P180 there is relatively high level of NMDAR1 expression in the V1 complex, with a slight variation in the intensity of staining detectable as a peak at P30 and a gradual slight reduction from P30 to P180. Around P40 a single row of intensely immunoreactive cells is seen along the line between the 2 main cell clusters. The V1 nucleus is uniquely outlined by its intense staining from P3 to P40 when it blends with the moderate subunit density seen throughout the mature thalamus. POm has a more gradual onset to peak densities at P21 that is always less intense than VPM. Intra-laminar nucleus, especially CL, peak even later at P35, retaining scattered cells that express high levels of NMDAR1 for up to 12 months. Overall staining intensity in the barrel cortex increases from P3 to P- 21, when staining appears more diffuse but to a transient expression of NMDAR1 by astrocytes, which appear to be the fibers as such the corpus callosum and fornix. At P30, NMDAR1 is only marginally lower than P21, but at P35 & P40 neurons in layers IV & VI show markedly decreased expression. Analysis of the most intensely labeled barrel cortex shows very intense staining at P3 & P7 which reduces through P14, P- 11 & P-30. The expression in these cells again goes up at P35 where they remain for as long as 12 months. These differences are currently being quantified by Western & Northern blots in a z hybridization. (supported by NS-13031)
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NMDARI subunits were localized with immunocytochemical methods in sections cut tangential to the cortical surface. In layer IV barrels, a strong reaction of NMDARI immunoactivity was created by the presence of a small number of densely labeled, large somata in the periphery of the barrel whose dendrites point toward the center of the barrel, and whose apical dendritic processes, roughly 1 μm in diameter, on their soma and dendrites, plus diffuse cytoplasmic labeling. Since NMDARI can form functional NMDA channels, the aggregates on cell membranes suggest that glutamate receptors are associated with glutamate receptor sites. These cell exhibit multiple dendritic morphology and few spines suggesting that they are aspiny, GABAergic, stellate cells. The distribution of these cells and their high content of NMDARI further suggest that they may be extraordinarily sensitive to changes in sensory activity levels arising from the whiskers and from other cells. These cells are surrounded by many other cells containing far lower levels of reaction product that is seen almost exclusively in their cell body. In addition to layer IV cells, each barrel contained a few clusters of densely labeled, small, circular profiles that we interpreted as cross-sections through some of the apical dendrite bundles of layer V cells. (supported by NS-25907)


Neurons in rat SI barrel cortex have been reported to develop biased receptive fields (RF) after days to weeks of experience with all but 2 whiskers trimmed on one side of the face ("whisker-pairing"). This type of adult plasticity or "bias" occurred in the superficial and deep layer neurons after only 24 hours of whisker pairing, a time prior to any change in layer IV neuronal RPs. These results challenge the question of whether early-born neurons in the superficial layers are required for the later layer IV plasticity. Epiphenal application of NMADA (150 μM for 15 min) under single whisker stimulation does not produce an increase in the field cortex. Fourteen days after the lesion, neurons of layers IV, V, VI still exhibit center (D2 whisker) and surround (D1, D3, C2 and E2 whiskers) RFs and evoked responses in nonanesthetized, trimmed whisker pairing 7 days after the cortical lesion, when all but 2 whiskers were cut on one side (leaving either D2 + D1 or D2 + D3). The trimming was repeated every other day for 5 days. On day 7 layer IV neurons in the D2 barrel failed to show the paired (ie., intact) whisker. In contrast, deep layer neurons in the same radial penetrations developed the bias. These results indicate that the superficial layers of SI cortex must be intact for normal plasticity in layer IV. We are studying now whether this apparent delay in layer IV plasticity is dependent on long periods of whisker pairing. (Supported by NS-25907)

CHARACTERIZATION OF A FUNCTIONAL WHISKER REPRESENTATION IN RAT BARREL CORTEX: OPTICAL IMAGING OF INTRINSIC SIGNALS VS. SINGLE-UNIT RECORDING. R.D. Frostig*, S.A. Markin, M.C. Koon, Dept. of Psychology, University of California, Irvine, CA 92717.

The whisker-to-barrel system provides the opportunity to stimulate an independent sensory unit in the periphery and measure the extent of its output in the cortex. To achieve this goal, we compared the functional representation of a whisker obtained with optical imaging of intrinsic signals to that obtained with single-unit recording. Specifically, we compared the area which exhibits stimulus and intrinsic signal responses to the area which exhibits stimulus-related neuronal responses.

We first determined the functional representation of the whisker in barrel cortex by imaging through a thinned skull during stimulation of an individual whisker for one second at 5 Hz. After imaging we removed the skull and performed depth spaced single-unit recordings guided by the imaged representation of the whisker while delivering identical whisker stimulation.

Although the largest change in the intrinsic signals are found within a localized area which we defined as the functional representation of the whisker (Masino et al., PNAS, 90: 9996-10002, 1993), it is a widespread area beyond the functional representation which exhibits a stimulus-related change in intrinsic signals. When we compared the area of the stimulus-related neuronal responses to the area of the stimulus-related intrinsic signal responses we found that the two were similar. Both response areas spread over a radial distance of approx. 2 mm from the center of the imaged representation. However, the spread of single-unit activity was not uniform: there was a significant decrease in single-unit responses starting at and extending beyond the border of the localized functional representation determined with imaging. These results demonstrate an extensive response area activated by stimulation of a single whisker as assessed by both techniques, and highlight the correspondence between intrinsic signals and underlying responses. Supported by the Beckman Foundation and NIH grant MH-50362.

EFFECTS OF WHISKER TRIMMING ON GABAa RECEPTOR BINDING IN DEVELOPING RAT WHISKER BARREL CORTEX: E. Salazar, S.C. Carpenter, R.L. Miller, T.A. Austing and J.J. Fuchs, Dept. Biological Sciences, University of North Texas, Denton TX 76203.

Sensory deprivation affects GABAergic systems in adult neocortex (e.g., Hendry et al. '90; Akhtar & Land '91), but little is known about the role of this permissive inhibitory system in shaping the developing neocortex. The present study investigates developmental effects of sensory deprivation on GABAa receptors in rat barrel cortex. Either the middle row or the outer rows of vibrissae were cut at P0. The middle row (P0) was relative to nondeprived barrel (P0.3). GABAa binding was low in immature (P0) and high in adult (P00-1) rats. When we compared GABAa binding between P0/P0(3) and P00-1, we observed a significant increase in GABAa binding in adult. We also examined GABAa binding in response to bicuculline and KA, and found that the GABAa binding was significantly lower in the adult rat than in the neonatal rat. These results suggest that GABAergic activity may play a role in sensory deprivation and that GABAergic activity may be important in shaping the developing neocortex. Supported by National Institutes of Health RO1-17542.


Neurons in rat SI barrel cortex have been reported to develop biased receptive fields (RF) after days to weeks of experience with all but 2 whiskers trimmed on one side of the face ("whisker-pairing"). This type of adult plasticity or "bias" occurred in the superficial and deep layer neurons after only 24 hours of whisker pairing, a time prior to any change in layer IV neuronal RPs. These results challenge the question of whether early-born neurons in the superficial layers are required for the later layer IV plasticity. Epiphenal application of NMADA (150 μM for 15 min) under single whisker stimulation does not produce an increase in the field cortex. Fourteen days after the lesion, neurons of layers IV, V, VI still exhibit center (D2 whisker) and surround (D1, D3, C2 and E2 whiskers) RFs and evoked responses in nonanesthetized, trimmed whisker pairing 7 days after the cortical lesion, when all but 2 whiskers were cut on one side (leaving either D2 + D1 or D2 + D3). The trimming was repeated every other day for 5 days. On day 7 layer IV neurons in the D2 barrel failed to show the paired (ie., intact) whisker. In contrast, deep layer neurons in the same radial penetrations developed the bias. These results indicate that the superficial layers of SI cortex must be intact for normal plasticity in layer IV. We are studying now whether this apparent delay in layer IV plasticity is dependent on long periods of whisker pairing. (Supported by NS-25907)

NONSPECIFIC SHORT-TERM ELECTRICAL STIMULATION OF A SINGLE FOREPAW DIGIT INCREASES THE PHYSIOLOGICAL REPRESENTATION OF THAT DIGIT IN LAYER IV OF RAT BARREL FIELD CORTEX: AN EXTRACELLULAR RECORDING STUDY. C.Y. Lee, R.S. Waters, C.A. McCandliss, E.J. Johnson. Dept. of Anatomy and Neurobiology, UT, Memphis, Col. of Medicine, Memphis, TN 38163

Limited differential sensory experience can alter the physiological organization of the glabrous representation of the forepaw in the forepaw barrel subfield (FBS) cortex in rat layer IV (see companion abstract, Waters et al.). To determine whether nonspecific short-term electrical stimulation can alter the physiological representation of the forepaw, we examined barrel cortex prior to and immediately after a short period of electrical stimulation.

Adult rats were anesthetized with Nembutal, head was stabilized, skull was opened over SI, dura was removed, and cortex was covered with warmed silicon fluid. Carbon fiber electrodes were used to map the representation of digit two (D2), D3, D4 prior to and immediately after electrical stimulation. Pulses of direct current (amplitude: 80-120μA, duration: 100 mssec, interval: 1 Hz) were applied to the tip of D3 for 2 hr. Following the period of electrical stimulation, the cortex was immediately remapped. The following results were obtained:

1. Following electrical stimulation, the area of cortex served by D3 increased by approximately 106.

2. These results suggest that brief periods of electrical stimulation are sufficient to immediately alter the physiological representation of the stimulated digits. These results bear directly on the role of plasticity in neocortex.

(Supported by USPHS GR NS-25824, NSF Grant BNS 88-02766)

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566.13 DIFERENTIAL SENSORY EXPERIENCE ALTERS THE REPRESENTATION OF THE FOREPAW IN FOREPAW BARREL SUBFIELD (FBs) CORTEX IN RAT. B.L. Water1, C.A. Le., R.C. Fowler2, C.A. McCaig, Dept. of Anatomy and Neurobiology, University of Tennessee, Memphis, TN 38163; 1Dept. of Psychology, Unis. of Mississippi, Oxford, MS 38677.

The relationship between the forepaw and the barrel-like structures in the forepaw barrel subfield ( FBs) in rat SI cortex offers an excellent model system to study the differential effects of sensory experience on cortical structure and function. We have investigated the physiological mapping and morphological analysis of FBs in rats trained on a sensory-motor task.

Adult rats, water deprived, were trained to depress a 9mm disk to receive sticklebarn food in a period of 18-30 days. Following training, animals were anesthetized with Nembutal, the head was stabilized, the skull was opened over SI, dura was removed, and the cortex was covered with warmed silicon fluid. Carbon fiber electrodes were used to map the forepaw representation in SI cortex in both hemispheres. Lesions were made to identify specific recording sites and to relate the electrophysiological map with the morphological map in FBs. Following physiological mapping, the cortex was removed, hemispheres were flattened and cut tangentially. Tissue was stained with cytochrome oxidase (CO) to obtain the results. The results were as follows: 1. Sensory-motor training resulted in an average increase of 24% in the area of cortex represented by glabrous digits tips in trained as compared to untrained hemispheres. 2. No morphological differences within the FBs were observed between trained and untrained hemispheres. 3. Limited sensory-motor experience is sufficient to alter the physiological map of FBs without a noticeable change in the morphological map in FBs.

(Supported by USPHS GB NS-23024, NSF Grant BNS 88-02766)


Earlier anatomical, biochemical and behavioral studies have shown that subject-environment interactions have significant effects on brain structure and learning capabilities. Moreover, it is well established that the cutaneous representations of the primary somatosensory (SI) cortex display a remarkable plasticity in response to environmental enrichment or deprivation through an experience-dependent process involving spatio-temporal cues. We have designed experiments to evaluate the influence of early differential tactile experience on the organization of the cutaneous "maps" of the SI cortex in the rat. Long-Evans female rats were housed under different tactile environments (standard, impoverished, enriched) for 1 month from the time of weaning. Their forepaw representation within SI was then reconstructed by using microelectrode mapping. The "standard" rats were housed in groups of 3, in a conventional environment. The " impoverished" rats were also exposed to a conventional environment, but each animal was housed in a separate cage. The "enriched" animals were housed in larger cages, in groups of 12 and were provided with objects of different shapes, sizes and textures. Preliminary results suggest that: 1) the skin territories more likely to be stimulated (prolephant skin surfaces) are best represented within the SI cortex; 2) early tactile enrichment through object manipulation and contact with mates improves the spatial resolution (smaller cortical receptive fields, RPs) of the cutaneous forepaw representation; 3) tactile impoverishment results in a degradation of the forepaw representation that is characterized by larger RPs, many double RPs, and by the emergence of non-cutaneous small "islands" within the cutaneous map. These results indicate that early tactile experience plays a critical role in the development and maintenance of the cutaneous cortical representations.

566.15 ACUTE CHANGES IN THE RECEPTIVE FIELDS AND NEURAL ACTIVITIES OF THE VPM-THALAMIC NEURONS DURING THE DEAFFERENTATION OF SINGLE DIGITS OF ANESTHETIZED RATS. A. V. Saganov1, P. DiSilvio1, A. H. Kiang2, A. B. Sininger1. 1Dept. Psychol, 2Vanderbilt Univ, Nashville, TN 37235 USA.

To investigate whether either temporary (TD) or permanent (PD) deafferentation processes recruitment of sensory maps in the forepaw area of the ventral posterior thalamus, we made single or multiple unit recordings from neurons VPM the thalamus of urethane anesthetized rats. Neuronal responses to subcutaneous electrical stimulation to the forepaw area, forepaw digit stimulation were quantitated by analyzing post-stimulus time histograms. After determining the minimal receptive fields or control period, either TD or PD of a RF (single digit) was carried out by ipsilateral injection 12% or amputation, respectively. Ten of 12 VPM units showed a reversible block of original RF (ORF) after TD and exhibited TD-like RFs. Nineteen units that belonged to neighboring neurons DPs were initially observed at 22.8 % of RF after TD and they disappeared at 57.3 % of RF after PD. The PD showed much weaker responses to the peripheral activation than ORF (determined by maximum 43.1-12.8% of the ORF RF). The final response latencies from the NRP (0.0-0.7-15.4-0.8 ms) were slightly longer than those from the ORP (7.1-0.8-15.4-0.8 ms). Seven VPM units were subject to PD study and they exhibited NRP sizes located anterior to the ORP. NRP sizes located anterior to the ORP (max. 96.8% of the ORP RF) were slightly larger than those from the ORP (96.8% of the ORP RF). Responses to the NRP of 0.0-0.7-15.4-0.8 ms were slightly longer than those from the ORP (96.8% of the ORP RF) (10.5-20.6 ms).

These results demonstrate that either temporary or permanent deafferentation of single digit induces a marked reorganization of the thalamic representation within minutes of VPM the thalamus. (KRP 93)

566.16 DEAFFERENTATION CHANGES THE DISTRIBUTION AND ORGANIZATION OF THE BURSTING PATTERNS OF NEURONS IN SOMATOSENSORY CORTEX OF AWAKE CATS. B.W. Dykes1, H.H. Webster, A.A. Myasnikov and I. Salimi. Dept. de physiologie and Centre de recherche, Dept de psychiatrie, Hopital Sac-oeur, Universite de Montreal, Montreal, Quebec H3C 3J7.

After identifying specific classes of neurons in the cat somatosensory cortex based on the structure of their spontaneous activity, these same classes were sought in somatosensory cortex that had undergone a large deafferentation by transection of the radial, median and ulnar nerves. Recordings made from several days to two weeks after the surgery showed an increased probability of encountering neurons that exhibited bursts (small cortical receptive fields, RPs) of the cutaneous forepaw representation or a decreased probability of encountering neurons with long bursts. This change suggests that deafferentation leads to less structure in the ongoing activity within deprived cortex. We hypothesize that this results from a reduction of the strength of corticocortical and corticobalamic feedback loops impinging on the deprived area.

(supported by the Medical Research Council of Canada)

566.17 DISTINCTIVE PATTERNS OF BURSTING IN NEURONAL ACTIVITY FROM SOMATOSENSORY CORTEX OF AWAKE CATS. A.A. Myasnikov1, H.H. Webster and B.W. Dykes. Department de physiologie, Université De Montréal, Montréal, Québec H3C 3J7.

Recent studies have shown that differences in the membrane characteristics of cortical neurons allow cortical neurons to be distinguished on the basis of the shape and temporal pattern of their action potentials. These characteristics may allow for the identification of a behaving animals. To examine this possibility in the somatosensory cortex, we obtained extracellular field recordings from neurons in awake, quietly resting cats using tungsten-in-glass microelectrodes. Neurons were characterized by their action potential waveforms, receive field characteristics and other electrophysiologic attributes. Analysis of the spontaneous activity showed some cells to have distinctive bursting patterns. Cells of one class had multiple peaks in their autocorrelograms, many impulses per burst and nearly constant interburst intervals. A second had only one peak in their autocorrelograms, short bursts, and increasingly longer intervals within the burst. Other cells lacked any temporal dependence between spikes. (funded by the Med. Res. Council of Canada)

566.18 NEOCORTICAL AND THALAMIC PLASTICITY FOLLOWING NEONATAL DEAFFERENTATION IN RATS. D.M. Li, J.T. Ross, J. Nisanov, and P.J. Hand. Institute of Neurological Science, University of Pennsylvania, PA 19104.

One critical issue involving activity-dependent neocortical plasticity is the relative contribution of intracortical and subcortical structures to representational changes. Our previous studies showed that neonatal removal of all whiskers except C3 produced a prominent enlargement of 2Dg functional representation of spared C3 (3C) in SI cortex. In this study, we used the same model to investigate the plasticity at the cortical and subcortical level. SC3 preparation resulted in a significant expansion of 2Dg functional representation in both SI cortex (O(3d), 3.35% 8%) and ventral thalamus (O(12.5), 3.4%) but not in the brainstem trigeminal complex. However, we only found a 99.5±17.6% enlargements in barre size as revealed by CO staining. Presumably the striking representational enlargement was only partly due to a structural growth. The use of the active whiskers in the passive whisker stimulation may involve in this additional expansion, we performed GAD immunochemistry in the sc3 model. Preliminary results showed that the pattern of GAD in somatosensory cortex is well organized and homogenized. In contrast, the boundary of each barre in deafferented barre cortex is disorganized and the GAD positive cell density of adjacent barres is significant attenuated as compared with sc3 region. These results suggest the GAD expression may be activity-dependent and its down regulation following deafferentation may reflect a decreased lateral GABAergic inhibition that contributes to the observed neocortical plasticity. [Supported by NIH-2P41RR01638].

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Somatotopic organization of the human somatosensory cortex was analyzed with echo planar imaging (EPI) at 1.5 T. All subjects were subjected to the deoxyhemoglobin as an endogenous contrast medium. Scrubbing stimulation at a frequency of 3 Hz was applied to one of three cutaneous areas: toes, fingertips, and tongue tip. Each recording session consisted of resting (40 s), stimulation (30 s), and resting (40 s) periods. Sagittal EPI slices of 8 or 15-mm thickness (data acquisition time, 60 ms per slice; in-plane resolution, 2 x 4 mm) were obtained every 2 s continuously in a single session. A spin echo image of the corresponding sagittal slice was taken just before and after several sessions. Statistical significance of signal changes was assessed with Student's t-test for each voxel. We found focal bands of increased signals (1.5 to 8 %) during stimulation periods, with rise time of 2 to 6 s. These activated bands were identified on the contralateral postcentral gyrus. The cortical responses from the three stimulation sites were anatomically distinct and organized mediolaterally in the order of toes, fingertips, and tongue tip.

567.3 MOTOROTOPIC ORGANIZATION OF HUMAN CORTICAL 20-Hz OSCILLATIONS. R. Salmelin, R. Hari, M. Hamalainen, and M. Kajola. Low Temp. Lab., Helsinki Univ. of Technology, 02150 Espoo, and Neuroimag Ltd., 00510 Helsinki, Finland.

In humans, the spectral distribution of cortical spontaneous rhythms (alpha, mu, tau) is dominated by frequencies around 10 and 20 Hz. The reactivity and source areas of these cortical somatosensory rhythms associated with self-paced left- and right-sided index finger, toe, and mouth movements were studied non-invasively in eight healthy subjects employing a 122-channel whole-head neuro-magnetometer. Following the complete suppression during movements, the rhythms manifested a pronounced increase above the baseline, particularly in the 20-Hz range. Sources of the enhanced 20-Hz activity were found in mesial cortex for toe movements and about 5 cm and 8 cm from Rolandic cortex (for hand and mouth movements, respectively, apparently tracking the representation of the moving body parts in the motor cortex. The reactive 20-Hz rhythms, on the contrary, were increased at about 6 cm from vertex, close to the hand area in the somatosensory cortex, for all tasks. The 10- and 20-Hz somatomotor rhythms seem to have separate spatial distribution and distinct functional roles in motor tasks, with the 20-Hz range manifesting motorotopic organization.


Respiratory related evoked potentials (RREP) recorded from C2-C3 and C2-C4 have been elicited by occlusion of inspiration. The initial peak positive (P1) is believed to represent the sensory cortical response to the activation of the afferents transducing the inspiratory load. It was hypothesized that the P1 peak amplitude is proportional to the inspiratory load magnitude in children. Children were studied semi-awake at rest. They inspired through a mouthpiece and non-rebreathing valve connected to a loading manifold. EEG activity was recorded from C2-C3 and C2-C4. The resistive (R) load magnitude was set to 21.0 cm H2O/sec (4.92KHz bandwidth). Evoked potentials were obtained by interruption of inspiration with the load, R, open, port open. The EEG activity was averaged on-line and stored on computer system. Each trial consisted of 80 load presentations. Each load magnitude and the control no-load were applied 20 times each in an trial. There were 4 trials. The P1 peak amplitude and latency were measured. No P1 peak was observed. The P1 also increased as the R load on loads and occlusion. The mean P1 latency were C2-C3=38.2, C2-C4=38.4 ms and there were no significant differences between R load magnitudes. The P1, were measured with the resistive (R) load magnitudes and for both electrode pairs. Inspiratory occlusion and resistive loads produce mechanical changes to the inspiratory transduced by respiratory mechanoreceptors. These data support the hypothesis that increased in inspiratory load are correlated with increased activation on sensory cortical neurons. Supported by ALAF grant UF #95005907.

567.2 PUTATIVE HUMAN SOMATOSENSORY AREA III (SIII) AND ITS MODULATION BY ATTENTION. J.V. Pardo, J. Lee. Psychiatry PET Unit, Psychiatry Service, VAMC, and the Division of Neuroscience Research, Department of Psychiatry, University of Minnesota, Minneapolis, MN.

PET and MEG have recently helped to define human SI (along the Rolandic Sulcus) and SII (parietal operculum). By analogy to the organization of non-human somatosensory cortices, Caselli has postulated the existence of human SII and SIII. Human SII and SIV are hypothesized to compose a ventrolateral somatosensory associative cortex whose damage causes tactile agnosia.

Ten healthy volunteers participated in a somatosensory processing task while cerebral blood flow was measured with the 11C-[1H]O Dihydroxybutane technique and PET. In the somatosensory attention condition, subjects monitored their finger, lip, or great toe for pauses in a series of taps (3 Hz) from a von Frey hair and maintained passive visual fixation. In the visual attention condition, subjects fixated attentively looking for rare, transient disappearances of the fixation mark. We recorded SII, right contralateral to the stimulatedips or contralateral toe, and lip responses in SII who were closely mapped together and were modulated significantly by attention.


Functional magnetic resonance imaging (fMRI) techniques have recently been developed to identify cortical areas activated during specific tasks. We have further modified these techniques to identify regions in the cortex activated during simple somatosensory and/or motor tasks.

All data were collected from normal subjects. Images were obtained with a 1.5 T MRI scanner. Each protocol typically consisted of alternating sets of 6 images during a task and 6 images at rest or during a control task for a total of 72 images. Typical imaging parameters were: 4mm axial cortical slice, TR=68ms, TE=40ms, bandwidth=4.92KHz, field of view=302mm, 256x128 matrix. flip angle=90°. Somatosensory-related events were presented to the patients during an electrical stimulus applied to the median nerve with a TENS-type stimulator to evoke strong perceptual awareness in the distribution of the nerve, or 2) natural tactile stimuli (e.g., light brush) of the skin. For comparative purposes, scans were also obtained during a sequential motor/sensory task (e.g., thumb to finger opposition with contact). Activated regions were determined based on a pixel by pixel statistical analysis (t-test) of the mean intensity signal difference during the task and control.

Natural tactile stimuli activated large areas of SII and SIII. Electrical stimuli that evoked paradiahic sensory activations disscnt areas of SII. During simple motor tasks, SII, SIII, and SIV were activated. Within activated areas, the mean signal intensity followed the expected somatotopic pattern indicative of cortical activation. These data indicate that fMRI can be used to identify and confirm the functioning of sensory and motor cortical areas active during simple tasks.


Functional magnetic resonance imaging (fMRI) has the potential to achieve greater spatial resolution than PET and SPECT. We tested the ability of fMRI to resolve individual finger representations within the somatosensory cortices (SI and SII) by applying a vibratory stimulus to single digits during a Tridimensional Trains or Stimuli (T) mapping protocol. In each session each vibratory and proprioceptive stimuli were applied to DII, DII, and DIII, 6.5 mm 6.5 mm 6 mm 6.5 mm stimulus-control cycles in each session. Five images/slice were obtained during stimulus or control. T-maps were superimposed on high resolution MR images. The vibratory cortical activation maps for all three digits showed significant activations (t-map clusters containing 9 or more pixels with all p<0.05) in SI and SII. The regions activated by DII and DIII were spatially distinct when measured both by the center of gravity of the peak which exhibited the largest increase in intensity within the activated cluster. In SI, the activated regions for DII and DIII were spatially closest while the activated regions for DII and DIII were more distant. The proprioceptive cortical activations were less localized than the vibratory activations.

This demonstrates that fMRI can distinguish the cortical representation of individual fingers in single subjects. Therefore, the spatial resolution of fMRI is higher than that of PET or SPECT.
567.7 SPATIAL MAPPING OF PAIN AND MOTOR ACTIVATION IN HUMAN CEREBRAL CORTEX WITH FMRI AT 4 TESLA. Michael J. Jaderala, Han Wei, Robert C. Coghlan, Steven D. Wolf and Robert S. Balaban. Neuromagnetobiology Branch, NEPR and Laboratory of Cardiac Energetics, NHLBI, NIH, Bethesda, MD USA.

A high resolution gradient recalled echo FMRI method was developed to visualize neural activity evoked by thermal nociceptive stimuli and motor tasks. Changes in neural activity reflected by blood flow and blood oxygenation level differences (in flow effects and T2 effect, respectively) were obtained by using 2 and 6 mm thick transaxial slices with repetitive 13 sec. scans that provided good contrast and were registered to high resolution anatomical scan. In both cases, activated voxels were discretely organized such that the activity generally traced the gyral borders and overlapped somewhat into the sulci. The appearance of the signal was consistent with tin originating from marginal or central gyral veins of medium caliber and possibly their collaterals. Mictalse acquisitions indicated that the nociceptive signal (from heating the tip of the thumb) occurred over 4-8 mm in the axial dimension and was contiguous between slices. The motor task (repetitive flexion of the last joint of the thumb) yielded a more extensive signal. For example, in 9 successive 5 mm slices starting from the superficial cerebral cortex to the level of the corpus callosum, contiguous activity (associated with the posterior bank of the precentral gyrus/superior sulcus) could be traced through adjacent 3 slices. One slice contained the major locus of activity. In the coronal plane the activity was very discretely distributed and these data suggested that a prominent portion of the signal increase arises from the venous compartment and that the signal provides functionally relevant and anatomically discrete information.

567.8 NEURAL BASIS FOR TACTILE ROUGHNESS PERCEPTION: THE RELATIVE CONTRIBUTIONS OF SLOWLY ADAPTING AND RAPIDLY ADAPTING AFFERENTS. D.T. Blakeslee, S.S. Hsiao, and R.O. Johnson. Krueger Mind/Brain Inst. and Dept. of Neuroscience, Johns Hopkins Univ. Baltimore, MD 21205

Psychophysical and neurophysiological studies were done to assess the relative contributions of slowly adapting (SA) and rapidly adapting (RA) peripheral afferents to tactile roughness perception. The stimuli consisted of 18 embossed surfaces composed of tetragonal dot patterns with constant, 3.5 mm center-to-center spacings, variable diameters (2.0, 100, 500, 2000 and 2500 mm) and variable heights (280, 350, 680 um). Dot diameter and height are shown to have differential effects on the responses of SA and RA afferents. It is postulated that differentially stimulating these afferents will allow us to determine the relative contributions of SA and RA afferents to roughness perception.

Psychophysical reports from 15 subjects show that both dot diameter and dot height have significant effects on roughness perception. Decreases in dot diameter and increases in dot height both cause increases in perceived roughness with dot height having a larger effect at small diameters: narrow dots feel more than 2 times as rough at 680 um than at 280 um, while wide dots feel equally smooth at all dot heights. Neurophysiological recordings from 12 SA and 14 RA afferents from the ulnar and median nerves of 3 Macaque monkeys were made using the same dot patterns. The neural responses, which are plotted as two-dimensional spatial event plots, are analyzed using a measure of spatial variation in firing rates (J.Neurosci. 12:3414-3426, 1992.). In accordance with previous results (see, Physiol. and Pharm., in press), only the SA afferents systematically match the psychophysics. While both SA and RA spatial variation measures are mildly affected by dot diameter, only the SA's are affected by dot height. These results strongly suggest that RA afferents do not contribute to tactile roughness and that SA afferents alone form the neural basis for tactile roughness.

567.9 TRANSFORMATION OF TACTILE SPATIAL FORM WITHIN A CORTICAL COLUMN IN AREA 3b OF THE MACAQUE. J. DiCarlo, S.S. Hsiao, and R.O. Johnson. Krueger Mind/Brain Inst. and Dept. of Neuroscience, Johns Hopkins Univ., Baltimore, MD 21205

Multi-electrode (7 elect., 400 μm spacing) recordings were made in area 3b from both hemispheres of the awake Macaque monkey. The electrodes were positioned such that isolated neurons had receptive field centers on the same spatial location of the monkey's distal finger pad. Typically this allowed simultaneous recording from 3-5 neurons along a perpendicular to the cortical surface, which we assumed to be within a single cortical column. The monkey performed a visual task while embossed letters and oriented bars were repeatedly scanned at a controlled force (30 μm) in 0.5-0.8 mm (electrode) across the distal finger pad. The locations of the penetrations were confirmed using standard histological techniques. Two-hundred and fifty neurons (65 recorded per animal) were studied using the letter and bar stimuli. The neural responses to the embossed letters were used to construct two-dimensional spatial event plots (SEPs). A structural analysis of the SEPs shows neurons recorded in the central layers tend to be more isomorphic (similar in structure to the stimulus letters) and more responsive (higher impulse rates) than neurons in both supra- and infragranular layers. Cross-correlation analysis on 223 simultaneously recorded pairs of neurons suggested monosynaptic excitatory connections between 34% and common input to 16% of the pairs. In general, among pairs exhibiting monosynaptic excitatory connectivity, the SEPs of the "sender" neuron are more isomorphic than those of the "receiver" neuron. The overall pattern of functional connectivity is consistent with anatomical studies; there are monosynaptic connections from the central layers of cortex to supragranular and infragranular layers and monosynaptic connections from supra- to infragranular layers. Together, these results support the hypothesis that local, columnar cortical processing of spatial form proceeds from layer 4 to supra- and infragranular layers and that this processing results in a significant transformation of spatial form.

567.10 SHORT- AND LONG-LATENCY SOMATOSENSORY EVOKED FIELDS REFLECT DIFFERENT ASPECTS OF TACTILE INFORMATION PROCESSING. N. Forsa, R. Salmelin, V. Jousetki and R. Hari. Low Temperature Laboratory, Helsinki University of Technology, 02150 Espoo, Finland.

We recorded whole-head somatosensory evoked fields (SEFs) from 7 healthy subjects to air puff (of dorsum of the middle finger) and electric (median nerve at the wrist) stimuli; the interstimulus interval (ISI) was 3 s. The early (20-60 ms) SEFs to both stimuli originated in the central/perietal somatosensory cortex (SI) and the late (>85 ms) SEFs bilaterally in the second somatosensory corticies (SII). An additional source was active at 70-110 ms in the contralateral parietal postcentral cortex (PCC) in the wall of the postcentral fissure. Only SI responses were markedly smaller in amplitude and longer in latency to airpuffs than to electric stimuli. In an odd-ball paradigm, electric or air puff stimuli were delivered to the thumb and 'deviants' (15%) to the little finger, or vice versa (SI 0.5 s). In SI, the deviants elicited similar responses irrespective of whether they were presented alone or among standards. In contrast, SEFs from SII, and especially those from PCC, were clearly larger to deviants presented without the intervening standards. The early and late SEFs apparently reflect different aspects of tactile information processing. SI and PCC seem to be more integrative and less stimulus-specific in function than the SI cortex.

567.11 VIBROTACTILE SENSITIVITY IN PATIENTS WITH CARPAL TUNNEL SYNDROME (CTS). C.M. Checkoski* and S.J. Balowinski, Institute for Sensory Research and Department of Biomedical Engineering and Neuroscience, Syracuse University, Syracuse, NY 13244

CTS is commonly diagnosed by neurologic examination and electrophysiologic evaluation. However, many investigators have recently advocated the use of vibrotactile testing as a complementary diagnostic tool. The purpose of this study was to determine if the idea that the large-diameter sensory fibers are affected first in nerve compression, possibly resulting in elevated vibrotactile thresholds. More recently, however, it has been shown that vibrotactile thresholds were elevated during coincident pain (Akgarian et al., Soc Neurosci Abstr 1992). The purpose of this study was to assess vibrotactile sensitivity in CTS quantitatively using strictly controlled laboratory techniques. Analysis on 223 simultaneously recorded pairs of neurons suggests monosynaptic excitatory connections between 34% and common input to 16% of the pairs. In general, among pairs exhibiting monosynaptic excitatory connectivity, the SEPs of the "sender" neuron are more isomorphic than those of the "receiver" neuron. The overall pattern of functional connectivity is consistent with anatomical studies; there are monosynaptic connections from the central layers of cortex to supragranular and infragranular layers and monosynaptic connections from supra- to infragranular layers. Together, these results support the hypothesis that local, columnar cortical processing of spatial form proceeds from layer 4 to supra- and infragranular layers and that this processing results in a significant transformation of spatial form.


Human subjects identified either the higher of two vibrotactile stimuli differing in frequency or the rougher of two tactile gratings differing in spatial frequency. Stimuli were presented under computer control by a feedback regulated vibrator or a servo operated surface stimulator previously described. On each trial stimulus pairs were separated by a ~0.5 to 30 second delay interval. For both vibrotactile and textured stimuli, percent correct decreased and reaction times increased as a function of increased delay interval length. Engagement in a distracting task was used to determine whether elevated thresholds correlate with the presence of pain. Detection thresholds were measured on 20 patients (36 hands) clinically diagnosed with CTS. Bursts of vibrotactile stimuli were presented to the affected hand (stimulus frequencies, 1, 10 and 300 Hz; conductor spaces, 0.08 cm² and 2.9 cm²). The subjects also rated the amount of pain that was present at the beginning of each testing session. Thresholds were compared to normative data in the literature and to a within-subject control site, the hypothenar. No elevations in threshold were found on affected areas in 19 hands during all testing sessions when compared to controls. Thus, the testing of vibrotactile thresholds for CTS may not be warranted. In 11 testing sessions elevation thresholds were obtained on affected areas where the subjects reported the presence of pain. These results indicate that elevated thresholds in CTS may actually be the result of the presence of pain. Supported by NIH, HD30098.
567.13


Two rhesus macaques performed a task that manipulated attention across tactile space and between somatosensory modalities. The task involved three concurrent stimuli: two tactile vibrations presented to the hands and an analogous auditory tone. The monkeys were cued in a blockwise fashion to discriminate between target and non-target stimuli at the three stimulus positions at a time. This enabled us to measure neural responses to physically identical stimulus arrays under conditions differing only by cue. An asymmetry between somatic allow us to separate the components of the response.

Psychophysical tests using 80% valid cues verified that the monkeys used the cue to direct their attention.

We surveyed 373 cells in areas 7R and 7b. Of these, 170 were held long enough for us to record replicate blocks under each attentional condition (for a total of 420 trials per cell), and 85 satisfied an additional F-ratio criterion representing task-relatedness and stability of response. Excitatory responses to the transient phases of the tactile stimuli were the most common response pattern, but there were notable exceptions. In SII, a subset of cells was excited by the sustained phases of the stimulus but inhibited by the transients. In 7b, bimodal tactile and visual responses were common, and a few cells showed consistent directional selectivity across modalities. Bilateral tactile receptive fields were found in both areas. In many cases, the response to the first stimulus was followed by a diminished response to the second. Temporal order effects may be related to the phenomenon of tactile masking. (Supported by NS 31060)

567.14


Cells in areas SII and 7R were observed under three different attention conditions: the "no attention" condition (blocks set 1 and 2), the "auditory stimulus" condition (blocks set 3 and 4), and the "auditory and visual stimulus" condition (blocks set 5, 6, and 7). The monkeys were cued to an auditory stimulus, with each treatment presented in replicated blocks of trials. 40% of the cells showed firing rate differences of 50% or greater between left and right targets, and a subset of cells were used for three stimulus presentations at a time. There was no consistency, however, in the directions of these effects. Across the population that we sampled, both spatial and cross-modal attention effects appeared.

Randomization analyses were applied to determine whether the magnitudes of these observed effects were greater than would be expected by chance alone. The paradigm for tactile stimulus presentation consisted of a set of blocks where the first was a blank block, the second was a cue block, and the third was a treatment block. Randomization procedures were used to assign the monkeys to one of the four treatment conditions, with the restriction that each treatment was compared to reference distributions generated by random assignment of treatment stimuli. These analyses showed that the observed attention effects were not significantly greater than would be expected by chance and, furthermore, that there was no significant serial correlation between observations within blocks. The assumption of independence of our observations made by conventional analyses of variance and t-tests is therefore inappropriate.

Analyses of variance using hierarchical error terms corroborated these findings. While a conventional analysis found 75% of the cells to show attention effects significant to p < .05, an analysis that made no assumption of independence within blocks found only 6% to be significant. Time series plots reveal random drifts in activity underlying the serial correlation effect. (Supported by NS 31058)

567.15

RESPONSES OF NEURONS IN PRIMARY SOMATOSENSORY CORTEX TO PREHENSION OF OBJECTS OF DEFINED GEOMETRY. E.P. Gardnee*, S. Ghosh, and C.E. Kopp, Dept. of Physiology and Biophysics, NYU Medical Center, New York, NY 10016.

These experiments measure the responses of cortical neurons when monkeys actively grasp and manipulate objects of defined geometry. The monkey is presented with a set of objects of specified shape (large and small spheres, cylinders, cubes and rectangular blocks) which he is trained to grasp and lift. Visual cues delivered on the screen of a video monitor indicate which knob should be grasped. The monkey's hand movements are videotaped using two genlocked cameras, and stored together with extracellular spike records of cortical neurons on a VHS video recorder. Quick freezes are made from VHS compressed video and electrophysiological data on a Macintosh Quadra computer, to allow frame-by-frame correlation of hand position with neuronal firing patterns. These experiments indicate differences in information processing in anterior and posterior portions of S-I cortex. In anterior S-I, where neurons have small receptive fields on single digits, the neuron's firing patterns correlate with hand position across the digit. These neurons appear most strongly activated when the edges of the cylindrical or rectangular knobs contact the receptive field, and are less responsive when the hand moves over the gently curved spherical surfaces. In posterior S-I, where receptive fields cover multiple digits, some neurons appear to differentiate the surface curvature of individual objects. Other neurons are silenced upon hand contact with the knobs, regardless of shape, and resume tonic activity when the hand is removed. We postulate that neurons in posterior S-I may play a role in global appreciation of the entire object, rather than in perception of specific features of single surfaces. (Supported by NIH: NS11852)

567.16

TWO TYPES OF VIBRATORY RESPONSIVE PACINIAN-LIKE NEURONS IN MONKEY PRIMARY SOMATOSENSORY CORTEX (SI) STUDIED DURING ACTIVE HAND MOVEMENTS. M.A. Lebedev, L.Nelson Department of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, 875 Monroe Avenue, Memphis, TN 38163.

Some SI neurons respond to vibratory stimulation of their receptive fields with vibration-entrained responses (Mountcastle et al., J. Neurophysiol. 32:452-469, 1969). Previously, we described the sustained and transient firing of neurons in SI activated by both raised and moving flats and extensions in response to palpation or passive movement of fingers. In a previous study, we evaluated a population of 18 SI neurons (L, 5.6, 20 and 3 from areas 3a, 3b, 1 and 2) and have been considered to be Pacinian-like because each responded better to the highest of the three vibrotactile stimulus frequencies. Two types of Pacinian-like SI neurons were found: (1) those that were strongly vibration-entrained (N=19), and (2) those that were not (N=12). Each neuron was tested for its response to vibration stimuli, with the vibration being increased to a constant level of intensity. Each stimulus was then repeated at three different intensities, and the neuronal response to each intensity was measured. The paradigm was that of a repeated measures design, with each neuron being exposed to different intensities of vibration. The most significant difference in firing was found at the highest intensity, where the neurons fired at the greatest rate. To rule out that the firing rate was due to increased input, the vibration stimulus was repeated at different intensities and the neuronal response was measured. The most significant difference in firing was found at the highest intensity, where the neurons fired at the greatest rate. To rule out that the firing rate was due to increased input, the vibration stimulus was repeated at different intensities and the neuronal response was measured. The most significant difference in firing was found at the highest intensity, where the neurons fired at the greatest rate. To rule out that the firing rate was due to increased input, the vibration stimulus was repeated at different intensities and the neuronal response was measured. The most significant difference in firing was found at the highest intensity, where the neurons fired at the greatest rate.
PAIN MODULATION: PHARMACOLOGY VI

568.1

INTRATHecal TREATMENT WITH ANTISENSE OLIGODEOXYNUCLEOTIDES TO THE NMDA-RECEPTOR SHOW EFFECTS IN NOCICEPTIVE TESTS IN RATS. M. Kuhlbeck, C. Alten, S. Pfeifer, D. O. Boss, C. R. M. H. Gasser, M. M. D. Granger, Control AB, Novum Unit, S-141 57 Huddinge, Sweden.

The NMDA receptor has been shown to be involved in transmission and modulation of nociceptive information in the spinal cord. Molecular cloning has recently revealed the existence of five different receptor subtypes termed NR1 and NR2A-D. Functional NMDA receptors can be reconstituted using the NR1 subunit in combination with any one of the four NR2 subunits. The different combinations differ in expression patterns in the CNS and in their pharmacological properties. We have studied the effect of receptor-specific antisense oligodeoxyribonucleotides (ODDN) to the NR1 subunit in rats.

METHOD: Rats (n=18) were divided into five groups receiving 5 pmol probe 1 (NR1 antisense 2) or probe 2 (NR1 antisense 1) Sallati 1985%. Two injections were made on day one, three and five. On day six, tail flick, hot plate and formalin tests were performed. The behaviour was scored by means of a computer program with five behavioral categories. When there was continuous wave 60 minutes after injection; rest and activity with/without paw protection and active pain behaviour (licking, biting). Number of paw jerks were also registered. After testing the rats were killed and the spinal cord was collected on dry ice for radioligand binding experiments. RESULTS: In the hot plate test the time latency was significantly prolonged in the antisense-treated group compared to controls. In the tail flick test the latency was the same although no significance was obtained. In the formalin test the total number of paw jerks was markedly reduced in the antisense group and active pain behavior was also affected. No motor disturbances were observed in any of the groups. Autoradiography with CGP37653 binding in spinal cord sections from all three groups was undertaken.

CONCLUSION: This study shows that antisense ODN may be a useful tool in understanding the role of different receptors and their subtypes in complex events such as spinal nociceptive transmission.

568.2

ROLE FOR NMDA RECEPTORS IN THE DEVELOPMENT OF MECHANICAL HYPERALGESIA PRODUCED BY CAPSAICIN IN RATS. H.L. Gilchrist and D.A. Simon. Neuroscience Research in Psychiatry, University of Minnesota, Minneapolis, MN 55455.

Intradermal injection of capsaicin (CAP) has been used as a model for cutaneous hyperalgesia in humans. When injected into the skin, CAP produced hyperalgesia to heat and mechanical stimuli. Furthermore, it was shown that hyperalgesia following CAP correlated with an enhanced excitability of dorsal horn neurons. In order to study underlying pharmacological mechanisms of CAP mediated hyperalgesia, a similar model was developed in rat and described previously (Suc. Neurosci. Abstr., 396, 1991). Briefly, rats received one intraplantar injection of CAP into the plantar surface of one hindpaw. CAP (1-30 µg) produced a dose-dependent decrease in withdrawal latency to heat as well as increased the frequency of withdrawal to mechanical stimuli (vibratory and brushing) by filament tests (vibratory and brushing) in quantified by filament tests.

In the present study, we investigated the role of spinal NMDA receptors in the development of hyperalgesia following CAP. A chronic intrathecal (I.T.) catheter was surgically placed at the level of the lumbar enlargement. I.T. pretreatment with the competitive NMDA receptor antagonist AP-5 (2, 4, or 8 µg in 5 µl) significantly attenuated the normally enhanced withdrawal responses evoked by mechanical stimuli without altering hyperalgesia to heat stimuli. This suggests that spinal NMDA receptors are necessary for the development of mechanical hyperalgesia following CAP in rat. It is also concluded that this model, hyperalgesia to heat may involve alternate mechanisms, such as sensitization of peripheral nociceptors. Data will be discussed in terms of the contribution of spinal NMDA as well as non-NMDA receptors to the development of hyperalgesia produced by CAP. Supported by NRSK DA05592 and the Minnesota Medical Foundation.

568.3

THALAMIC N-METHYL-D-ASPARTATE (NMDA) RECEPTORS ARE INVOLVED IN THE DEVELOPMENT AND MAINTENANCE OF HYPERALGESIA FOLLOWING UNILATERAL HINDPAW INFLAMMATION. K. Kohlhaas*, and O.F. Gabbott. Dept. of Pharmacology, Univ. of Iowa, Iowa City, 52242.

Effects of intrathalamic injection of an NMDA receptor antagonist, D-2-amino-5-phosphonovaleric acid (D-APV) on the thermal and mechanical hyperalgesia induced by hindlimb intraplantar injection of carrageenan in the rat were studied in the "acute phase" (within 3 hrs) and the "subacute phase" (24 hrs) after carrageenan administration.

Male Sprague-Dawley rats were injected in the left hindpaw with 2mg carrageenan. Noxious mechanical and thermal hyperalgesia within 3 hours of injection. Two consecutive doses spaced 30 min apart of either D-APV (5 nmol) or saline were injected into the hindlimb representation of the ipsilateral thalamus (0.5 µl).

Treatment with D-APV, but not saline, in the contralateral thalamus significantly reduced both the acute mechanical and thermal hyperalgesia induced by carrageenan. There was no effect on the non-inflamed hindpaw. Rats treated with D-APV in the acute phase failed to develop thermal and mechanical hyperalgesia in the subacute phase. In contrast, rats treated with saline or D-APV in the ipsilateral thalamus in the acute phase demonstrated significant thermal and mechanical hyperalgesia in the subacute phase.

Taken together, these results suggest an involvement of thalamic NMDA receptors in the development and maintenance of hyperalgesia associated with inflammation in a model of chronic pain.

568.4

NMDA AND NK1 RECEPTOR ANTAGONISTS ACT ADDITIVELY TO REDUCE BEHAVIORAL HYPERALGESIA IN RATS WITH UNILATERAL INFLAMMATION. K. Ren* and R. Dubner. Neurology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Previous studies suggest that both N-methyl-D-aspartate (NMDA) and tachykinin (NK1) receptors are involved in spinal nociceptive transmission. To assess the interaction between NMDA and NK1 receptor systems on nociceptive responses of rats with complete Freund's adjuvant-induced hindpaw inflammation/hyperalgesia, an isobolographic analysis was performed using competitive antagonist tests against NMDA or NK1 receptors. Thermal hyperalgesia, determined by the ability of a noxious stimulus to elicit a reflexive withdrawal response, was induced by a single injection of complete Freund's adjuvant (1:1 emulsion, 100 µg Mycobacterium) into the hindpaw of rats. The drugs were given intrathecally. Dextroprophan, a non-competitive NMDA receptor antagonist, attenuated hyperalgesia dose-dependently with a maximal ~80% reversal of hyperalgesia. WIN-51,708 and CP-96,345, a combination of Dextroprophan and WIN-51,708 was then given at a fixed ratio. The ED50 value obtained from co-administration of the drugs was found to be not significantly different from the isobolographic line generated by individual ED50 values of the two receptor antagonists. These results suggest that although both NMDA and NK1 receptor systems play a role in inflammation-induced hyperalgesia, the two systems appear to act independently.
568.5
Supraspinal morphine analgesia in the peri-aqueductal gray (PAG) is modulated by mu and delta opioid receptors and by SHT, and SHT, receptors in the rostroventral medulla (RVM). Since NMDA and muscarinic receptors in the RVM have been implicated in nociception, the present study evaluated whether NMDA and mementic (scopolamine, pirenzepine) antagonists in the RVM altered PAG morphine analgesia on the tail-flick and jump tests. PAG morphine (2.5 μg) analgesia was dose-dependently reduced by MK-801 (0.03-3 μg: 50-100%) and AP7 (0.01-1 μg: 30-70%) in the RVM on both tests. RVM injections of MK-801, but not AP7 reduced basal nociception. PAG morphine analgesia was dose-dependently reduced by the muscarinic antagonist, scopolamine (0.5-5 μg: 70%) and less so by the M₁ antagonist, pirenzepine (0.05-5 μg: 35-50%). Both muscarinic antagonists reduced basal latencies, but not nociception thresholds. These data implicate NMDA and muscarinic receptors in the complex modulation of PAG morphine analgesia by the RVM.

568.6
Excitatory Amino Acids (EAAs) are contained in and released from nociceptive primary afferents. Although EAAs are apparently contained in all nociceptiveafferent types, the EAAs receptors that mediate the nociceptive responses produced by activating these afferents have not been determined.
In this experiment, we determined the effects of intrathecal (i.t.) administration of the NMDA and non-NMDA antagonist DNXQ on nociception produced by activation of Aδ or C nociceptors. Administration of APS produced a selective, dose-dependent increase in latency. paw foot withdrawal responses evoked by low nocuous skin heating rates, but did not alter nociceptive responses produced by high skin heating rates in doses that did not alter motor activity. These results indicate that activation of C-nociceptors produces nociception mediated in part by NMDA receptors. In contrast, intrathecal DNXQ increased latencies for both high and low heating rates. These results suggest that EAAs released from both Aδ and C nociceptors act on non-NMDA receptors to produce nociception. Supported by USPHS Grants DA08256 (DOV) and DA03880 (HHP).

568.7
Organic variables have been shown to play a modulatory role in the expression of endogenous pain control mechanisms. This study assessed lifespan changes in the magnitude and neurochemical mediation of cold-water stress-induced analgesia (SSIA) in male and female young and old Swiss-Webster mice. We have previously shown that this swim stressor produces enhanced analgesia on the hotplate test in both male and female mice, and this analgesia is mediated by the NMDA receptor in young adult males, but not in similarly aged females (Mogil et al. Pain. 53, 17-25, 1993). In the present study, male and female young and old mice (approximately 15 months of age) mice were tested for their analgesic response to a single stress exposure in warm water maintained at 33°C. In 32 day-old animals, male and female mice exhibited analgesia following the swim stressor that was antagonized by MK-801 (0.075 mg/kg, ip), and was therefore NMDA mediated, in both sexes. Thus, puppafemales display primarily NMDA-mediated SSIA, whereas we previously reported that sexually mature females do not (Mogil et al., ibid.). In 15-month-old animals, MK-801 did not antagonize SSIA in either sex. Thus, a qualitative difference in SSIA mediation noted across the lifespan is in male mice, as well as females. Analgesic magnitude increased significantly in male mice with aging, but not in females. These findings indicate that ontogenetic stage can contribute significantly to the expression of SSIA, and should be taken into consideration in future studies on SSIA mechanisms. This research was supported by NIH grant NS07629 and an Unrestricted Pain Research Grant from the Bristol-Myers Squibb Company.

568.8
PERIPHERAL ADMINISTRATION OF EXCITATORY AMINO ACIDS MODULATE NOCICEPTIVE BEHAVIOR IN RATS. D.L. Jackson, J.D. Richardussen, R.M. Haggrensw, Dept. of Rest. Sciences and Pharmacology, University of Minnesota, MN 55455.
While it has been demonstrated that certain primary afferent neurons in a neonatal rat spinal cord-cord preparation are activated by peripherally administered glutamate (Auil and Hildebrand, Agents Actions 1993; 39:C143-144), relatively little is known about the peripheral actions of EAAs in the intact adult animal. We aimed to examine possible peripheral mechanisms of nociception modulation by EAAs. Male Sprague-Dawley rats were tested for thermal hyperalgesia by placing them in a clear plastic chamber with an infrared heat source aimed through the glass floor of the chamber at the treated hindpaw (Pain 1986; 26:77-88). Paw withdrawal latencies were detected by a photocell and recorded by an observer blinded to treatment allocation. Following the collection of basal latencies, rats received 100 μl ipl injections of L- or D-glutamate (10 nmol), aspirate (100 nmol), or saline vehicle. Post-injection latencies were measured at 15 and 60 minutes. Data were analyzed by ANOVA and Duncan's test and expressed as means±SEM. L-glutamate reduced latencies at 15 minutes relative to the saline control (-10.0±15.6% vs. +5.3±15.9%; p<0.05). The stereoseomer, D-glutamate, had no effect on latencies at this time point (+10.6±13.7%). This is in contrast to the observed increase in latency at both 15 and 60 minutes following the ipl injection of aspirate relative to control (15 min = +4.2±11.4% vs. +4.6±11.9%; p<0.05, and 60 min = +5.0±10.6% vs. 1.8±11.9%; p<0.05). Collectively, these results support the hypothesis that the glutamate and aspartate can differentially modulate nociceptive behavior when injected into normal tissue. This research was funded by K16-DE02027, DE08860, DE10003, and a Howard Hughes Medical Institute Predoctoral Fellowship LDRD.
568.11
PAIN REDUCTION BY INTRATHRAL ADMINISTRATION OF GLYCINE.
Neurosurgery Department, Baylor College of Medicine, Houston, TX 77030.
Glucine, the primary inhibitory neurotransmitter of the spinal cord, is found in
high concentration within segmental interneuron pools. Glucine is released during
excessive spinal afferent stimuli, an effective means of nociceptive pain.
We hypothesized that the pain is attenuated by glucine.
Nociceptive rats were created by unilateral partial ligation of the proximal sciatic
nerve with 4 absorbable chronic sutures placed 1 mm apart. A PE-10 catheter
was introduced into the cisterna magna and directed towards the lumbar enlargement.
Twelve days after PE-10 implantation, rats were selected based on the induction of
nociceptive behavior, monitored by intrathecal glucine, MK-801, and 5,7-DKA, at a concentration of 0.1 µmol for 2 hours and
at a rate of 5-10 µl/h. Pain scores were calculated using the Randall-Selitto
scale of 0-4. The effects of glucine, MK-801, and 5,7-DKA on nociceptive behavior
were measured and compared to sham surgeries. Our results demonstrated that
the force necessary to produce nociceptive behavior could be significantly
lowered by the administration of glucine. This suggests that glucine may be
a potential therapeutic agent in the treatment of chronic pain.

568.12
ROLE OF NMDA RECEPTOR AND NITRIC OXIDE (NO) IN THE FACILITATION BY INTRATHAL (IT) NMDA OF LIMB WITHDRAWALS EVOKED BY GRADED NOCICEPTOR STIMULATION. L. Cantell*, Neurobiology, Physiology & Behavior, Univ. of California, Davis, CA 95616.
Secondary hyperalgesia is thought to be mediated by hyperexcitability of nociceptive spinal neurons via (a) glutamatergic overactivity of the NMDA-ionotropic
NMDA receptors to (c) allow Ca2+ influx to (d) effect persistent cellular changes in
behavioral studies, hyperalgesia is thought to be mediated by hyperexcitability
of nociceptive responses such as wind-up. We investigated if IT NMDA receptors
threshold and threshold responses using a quantitative measure of limb withdrawal response (LWR) magnitude, and if this is prevented by NMDA receptor antagonists (APV, MK801, respectively) or the alternate substitute for NO synthase (L-NAME).
One wk after implanting IT catheters, the ventral lip was fixed to a thermometer to
determine an ascending series of heat pulses (30°C-50°C, 15 sec per pulse).
The normalized area of integrated limb flexor EMG gave a measure of WRT magnitude across
stimulus-response function (SRF). Two paradigms were used: (1) One SRF was generated with a 60°C no drug (control), (b) 3 NT IT NMDA (100 µmol) given IT prior
to each heat stimulus, or (c) 10 µl of IT APV (5 µmol-µl), MK801 (1 µmol) or L-NAME (1 µmol) followed by (d). Two SRFs were generated, the first with no drug (control) and the second 20 min later with (b), (c), (d) each heat stimulus preceded by IT NaCl (0.9%), or (e) APV, MK801 or L-NAME followed by (d).
In the 2-SRF paradigm, NMDA significantly increased WRT magnitude in 40-49°C.
controls, reducing the SRF threshold and slope. This was not prevented by APV,
MK801 or L-NAME. In these cases where NaCl or MK801 = NaCl was given,
the second SRF was shifted toward significantly higher temperatures. Thus, WRTs appear
to be sensitized during the second SRF. This sensitization may confound any effect of
NMDA on the second SRF.
In the 1-SRF paradigm, NMDA significantly reduced the threshold of the SRF
compared to controls. This was prevented in rats pretreated with APV, MK801 and
L-NAME. These data support the idea that the NMDA receptor and NO are involved in
the development of hyperexcitability in spinal neurons mediating the WR.

568.13
SPINAL STRYCHNINE INCREASES RESPONSES OF NOCICEPTIVE DORSAL HORN NEURONS TO LOW THRESHOLD INPUT AND LENGTHENS INTRADORSAL DISCHARGE. L. Sekontola* and J. Robertson, Anesthesiology Research Lab, Univ. of Calif. @ San Diego, La Jolla CA 92033-0188.
Hyperesthesia following many noxious injuries is associated with inhibitory interneurons loss. Pharmacological blockade of excitatory receptors in spinal cord
nociceptive dorsal horn neurons.
Excitatory properties of spinal neurons (STN) results in tonic evoked
alldynia. It has been proposed that this is due to loss of "auto-inhibition" of
low threshold (LT) input to wide dynamic range (WDR) cells via an inhibitory
input. This study determined if spinal strychnine (STN) results in excitation
responsiveness of dorsal horn neurons.
Dialysis probe was inserted ventral horn of chloroento-anesthetized cats and
electrocellular recordings made from WDR cells in close proximity to the probe.
Artificial CSF was perfused and baseline responses to a range of mechanical stimuli
measured. The perfusate was exchanged to STN (1 µM) or an NMDA antagonist
(2 mM APV) or STN + a non-NMDA antagonist (1 mM CNQX) and the receptive
field properties measured.
Exposure to STN resulted in a greatly enhanced high frequency response to LT
input (especially hair). The firing pattern was usually one of irregular bursts.
The response to high threshold input increased, but to a lesser degree. The high
threshold response was usually followed by a prolonged afterdischarge (several
minutes). This was less seen less frequently after LT input. Receptive field size also
increased substantially. In most cases, basal firing rates were unaffected.
These results indicate that this enhanced responsiveness was blocked by co
administration of APV and CNQX reduced evoked activity while leaving the
afterdischarge relatively intact.
These results support the hypothesis that removal of tonic glycine inhibition leads
to excessive activity in WDR neurons which is mediated by activity at a local
NMDA receptor. (This study was supported by NS 11255)

568.14
Excitatory amino acids, via activation of the NMDA receptor, are implicated in
the persistence of pathological pain. Recent work in our laboratory indicates that the transplantation of adrenal medullary chromaffin cells into the spinal cord
subarachnoid space can alleviate chronic pain syndromes in animal models. The
purpose of this study was to determine whether adrenal medullary transplants act
via modifying spinal NMDA hyperexcitability. For this study intrathecal catheters
were implanted into the spinal subarachnoid space of rats. Rats also received
either adrenal medullary transplants or controlistrated muscle transplants via
laminectomy at the level of the lumbar enlargement. Abnormal pain responses
to several doses of intrathecally administered NMDA were determined using
behavioral tests for plantar allogeneic and mechanical and thermal hyperalgesia.
Results indicated that NMDA produced allogeneic and hyperalgesia and control
transplanted animals in a dose related fashion. In contrast, the dose response to
NMDA was significantly shifted rightward in animals with adrenal medullary
transplants, and hyperalgesia and allodynia were completely alleviated in the
lower dose ranges of NMDA in these animals. The beneficial effects of the
transplants were not decreased by naloxone, but were partially attenuated
by phenolamine, indicating a limited role for catecholamines released by the
transplanted cells. However, this did not completely account for the effects of the
transplants. In summary, these results suggest that adrenal medullary
subtransplants may intervene in the cascade of hyperalgesia initiated by
the activation of NMDA receptors in pathological pain syndromes. Supported
by NIH grant NS25054.

568.15
Dextromethorphan (DM) is a clinically available oral antitussive and NMDA receptor antagonist with the ability to attenuate neuronal "wind-up" (Dickenson et al., 1991). We have found that pretreatment with DM (6, 20 or 60 mg/kg sc) suppresses formalin-induced nociceptive behaviors in a dose-dependent manner. Nociception turns on the expression of the immediate early genes (IEG) including c-fos and c-fos like genes during inflammation. In 30 minutes post injection. No change in c-fos mRNA was detected. The contralateral S2H, nucleus raphe magnus, periaqueductal gray, thalamic thalamus, or motor cortex. Pretreatment with DM at 60 mg/kg sc 30 minutes prior to formalin resulted in a suppression of c-fos induction. This suggests that c-fos mRNA levels in S2H of animals receiving DM prior to formalin did not differ from controls. These data indicate that DM suppresses formalin nociceptive behavior and one of the biochemical consequences of formalin nociception, i.e., induction of c-fos mRNA. Supported by the VVZ Research Foundation (JKE) and DA01457, DA01724, DA0196 and CA2897.

568.16
Slow temporal summation of second pain is thought to be a psychophysical
correlate of temporal summation of C-afferent-mediated responses of
dorsal horn nociceptive neurons, termed windup. Windup has been shown to be
mediated by N-methyl-D-aspartate (NMDA) receptor activation within the
spinal cord of experimental animals. We thus tested whether a NMDA receptor
antagonist would reduce slow temporal summation of second pain in
man. Oral doses of dextromethorphan (DM), a common cough suppressant
and NMDA receptor antagonist, and their vehicle control were given on
a double blind basis to normal volunteer human subjects who rated intensities
of first and second pain in response to repeated painful stimuli separated
by 30 seconds (mean 5°C) and repeated 32°C heat pulses. Doses of 30 and 45 mg but not 15 mg (in
a single bolus) were effective in attenuating temporal summation of second pain as
compared to vehicle controls (Wilcoxon signed-ranks test, P < 0.03). By
contrast, neither first pain nor second pain evoked by the first stimulus in
a train of stimuli were affected by any of these doses of DM (P > 0.05). These
results further confirm temporal summation of second pain as
a psychophysical correlate of windup and provide evidence that DM
selectively reduces temporal summation of second pain, as has been shown for
windup.
568.17
Repetitive stimulation of somatic nociceptors evokes progressive increases in the excitability of spinal neurones ("wind up"). Some forms of nociceptive stimulation may induce excitatory changes in the last-order potential of somatic reflexes. These changes are believed to be mediated by the activation of NMDA receptors on spinal neurones. In this study we have investigated the excitatory changes induced by nociceptive viscerosomatic reflexes and the role of spinal NMDA receptors in their generation.
Experiments were conducted on decerebrated and spinalised rabbits. Reflex activity was elicited by a lumbar nerve in response to supramaximal electrical stimulation of the ipsilateral second lumbar nerve (somatic reflex) or of the sciatic nerves (viscerosomatic reflex). Changes in excitability of these reflexes during repetitive stimulation at 1Hz ("wind up") and up to 5 minutes after (reflex potentiation) were studied as were the effects of intraventricular administration of the NMDA receptor antagonist ketamine at doses between 1 and 30 mg/kg.
"Wind up" was consistently evoked in somatic reflexes but less so in viscerosomatic reflexes. Potentiation of viscerosomatic reflexes was rarely seen and often these reflexes were depressed by repetitive stimulation of visceral afferents. Ketamine caused a dose-dependent decrease of both somatic and viscerosomatic reflexes and a reduction in the magnitude of "wind up". We conclude that there are important differences in the spinal organization of somatic and viscerosomatic nociceptive reflexes and that NMDA receptors influence the baseline levels of reflex excitability in the spinal cord and as the dynamic components of the reflexes.

568.18
BEHAVIORAL AND ELECTROPHYSIOLOGICAL EFFECTS OF MEMANTINE IN A PRIMATE MODEL OF PERIPHERAL NEUROPATHY. S.M. Carson, R. Rest, K. Gordon, M. Cope, G. W. Moore, and W.D. Willis. Dept. of Anatomy and Neurosciences, Marine Biomedical Inst. UTMB, Galveston, TX 77555
The development of animal models mimicking painful peripheral neuropathies allows investigation of the underlying mechanisms that will be used to improve clinical treatment. NMDA receptor activation is an important mechanism contributing to neuropathic pain. Memantine (MEM), a non-competitive NMDA antagonist, in attenuating behavioral and electrophysiological abnormalities previously documented in a monkey model of peripheral neuropathy.
In an anesthetized monkey (Macaca fascicularis), on L7 spinal nerve was tightly ligated, a sham operation was performed on the contralateral side. Compared to presurgery levels, the animal demonstrated an increased sensitivity to mechanical stimulation (allogynia) with von Frey hairs and brushing with a cotton ball. This was most apparent on days post-surgery. On day 36 post-surgery, MEM was delivered in a bolus infusum (3 mg, 10 mg, subcutaneous (12mg) or orally (30 and 50mg). The first test period of each test day the animal was assessed for withdrawal reflexes toward a range of stimuli (painful and non-painful). The second test period was repeated at hourly intervals. The most effective route in attenuating allodynia in the EXP group was for oral administration; the subcutaneous injection did not have any effect. The response on the control foot were not affected by any route. The results suggest that MEM is effective in a peripheral neuropathy model.

568.19
SYSTEMIC TREATMENT WITH MEMANTINE REDUCES MECHANICAL ALLODYNNIA IN A RAT MODEL OF PERIPHERAL NEUROPATHY. G. Hargens, A. Friedman and L. Hruby. Dept. of Anatomy and Neurosciences, Marine Biomedical Inst. UTMB, Galveston, TX 77555
A recently developed animal model of peripheral neuropathy is characterized by mechanical allodynia which develops within days following surgery and lasts for 3 months following resolution of the injury. The goal of the present study was to determine whether treatment with the non-competitive NMDA antagonist Memantine (MEM) had therapeutic and/or prophylactic effects in relieving mechanical allodynia observed in this animal model.
Acute delivery: In 3 groups of rats (Sprague-Dawley, 125-175g) a dose-response curve was established. Following baseline testing with von Frey hairs on the ventral surface of each foot and performance on a rotarod, animals were anesthetized and the L5 and L6 spinal nerves ligated with 0-5 suture. Animals were retested on days 1, 3, 5, 7, 9, 12, 15, and 17 post-surgery. The pumps delivered MEM at a continuous rate of 4mg/kg for 4 weeks. To determine prophylactic effects, rats were implanted with IP pumps day 2 prior to surgery. At the end of the experiment all rats (n=10) were administered a dose of 0.1 mg/kg MEM for withdrawal response on von Frey hairs and rotarod performance.
Acute IP injections of MEM demonstrated that saline and 0.1mg/kg MEM had no effect on behavior. 5mg/kg MEM significantly attenuated mechanical allodynia at the post-injection with no motor impairment. Animals receiving 10mg/kg MEM effectively attenuated mechanical allodynia at 1, 2, 4, and 8hrs, the animals displayed hypothermia. No effect was observed on the contralateral control paw. Preliminary studies concerning chronic treatment with 4mg/kg via pumps (n=3) demonstrated a therapeutic effect with no abnormal motor signs. However, this dose had no obvious prophylactic effect. Results indicate that NMDA receptors play an important role in the expression and in MEM may be a successful treatment for neuropathic pain. (Supported by NS11255 and NS27910.)

568.20
The sequence of events that leads to neuropathic pain may include activation of the N-methylD-aspartate (NMDA) receptor-ion channel complex since pretreatment with compounds as well as noncompetative NMDA receptor antagonists prevents nerve injury-induced abnormal pain. Earlier studies in our laboratory have shown that spinal adrenergic mediator transplants reduce neuropathic pain over an extended period of time. The effects of adrenalin transplants on spinal NMDA receptor binding was investigated using a new high-affinity glutamate binding site antagonist, CUP-39653. Saturation studies in spinal cord revealed that [125I]CUP-39653 binding affinity in control cerebral cortex but even lower. Neuropathic pain was induced in rats by loss of ligation of the right sciatic nerve (Bennet and Xie, 1990). Two weeks following surgery, either adrenergic medullary or sham transplanted rats were sacrificed in the subarachnoid space above lumbar spinal cord. One week following implantation, membrane preparations were made from spinal lumbar regions L5-L6, the spinal cord split in half and the dorsal central and homogenized septic extract. Animals that received control transplants showed decreased [125I]CUP-39653 specific binding ipsilateral to nerve ligation, compared to the contralateral side and unoperated controls. In contrast, adrenal medullary transplants bilaterally increased [125I]CUP-39653 binding, bringing binding on the nerve-injured side back to near normal control levels. These results suggest that adrenergic medullary transplants reduce neuropathic pain by equilibrating abnormal excitatory amino acid transmission in the spinal cord. Supported by NIH grant NS20504.

569.1
We will state and discuss the proposition that cortical evoked potentials are generated when neurones interact with glial cells across extracellular space. The evidence is as follows. First, glial research by Kuffler, Nichols, Dowling, and dozens of others confirms that the claim that the major evoked potential of the eye, the retinal b-wave, is produced this way. Second, the b-wave of freely-moving rats (stimulated through a light-emitting diode implanted above an eye) approximately doubles in amplitude during slow wave sleep; similar plastic changes take place in the visual cortical evoked potential response. Third, when the rat receives paired flashes, the refractory periods measured for b-wave and for cortical responses are both prolonged, but not identically, b-waves begin when synaptic potentials generated by rods and cones initiate potassium currents within Müller (glial) cell cytoplasm. The evidence that cortical astrocytes similarly optimize [K+]o is still indirect, but nothing uncovered so far seems to rule the possibility out.

569.2
SCLERAL DISTRIBUTION OF ERG B-WAVE AND OSCILLATORY POTENTIALS (OPs). P. Lachapelle*, J. Benoit* & C. Casanova*. Dept. of Ophthalmology, McGill University - Montreal Children's Hospital, Montréal, Que., Canada H3C 1P3 and Université de Sherbrooke, Sherbrooke, Que., Canada J1H 5N4.
Previous studies reporting the scleral distribution of the ERG made use of a bipolar recording approach which could have masked the existence of localised ERG dips. We investigated this issue in (n=10) anesthetized, paralyzed and artificially ventilated New Zealand rabbits. Scleral ERGs (1,000Hz OPs and 100,000Hz OPs) were recorded with a 0.5 mm tungsten electrode with reference and ground electrodes positioned in the mouth and neck muscles respectively. The anterior portion of the globe was dominated by a posterior b-wave whose amplitude decreased over the inner cornea. It was gradually replaced by a slow negative wave (inversion at -5-6 mm posterior to the limbus) onto which were superimposed large oscillations. These new ERG components were recorded in bipolar recordings, reaching a maximum at 7-mm posterior to the limbus. FFT analysis performed on this scleral location indicated that: 1- the negative wave is not an inverted b-wave, 2- the large OPs are generated in the large OPs (CUP-39653 some 7-8 mm posterior to the limbus. Our results indicate that the OPs are the most consistent retinal potentials since they are found at all scleral locations while the b-wave appears to be limited to the anterior portion of the eye. Furthermore in showing that maximal OPs are obtained in b-wave free locations suggests that the two retinal generators (b-wave and OPs) may interact in an inhibitory fashion. Funded by MRC MT12153.

RETINA AND PHOTORECEPTORS VI
STIMULATION OF RETINAL DOPAMINE D2 RECEPTORS AFFECTS AMPLITUDES OF THE B BUT NOT A WAVES OF THE RABBIT ELECTRORETINOGRAPHY. L. Bourguet, D. Belliveau, F.D. Jolicoeur* and C. Casanove. Departments of Ophthalmology and Physiology-Biophysics and Departments of Psychiatry and Neurology* Faculty of Medicine, Univ. of Sherbrooke, Sherbrooke, Quebec, Canada J1H 5N4

The two classical sub-types of dopamine receptors, D1 and D2, are present in the retina. We have shown that stimulation of both receptors with the mixed D1 and D2 agonist apomorphine results in marked reductions in amplitudes of both a and b waves of the rabbit ERG. In order to better delineate dopamine receptor responsivity to these stimuli, we have examined the effects of N-acetylcysteine (NPA), a purported selective D2 agonist, on retinal electrophysiological responses. Cells were isolated from anesthetized adult pigmented rabbits. A corneal electrode was inserted near the limbus to record the ERG. Responses were evoked by a diffuse flash (Grass PS2) and averaged 25 times. Three intensity levels were tested. NPA was injected intravenously at various concentrations (0.01-10 μg in 100 μl). Our results indicate that the intravitreal injection of NPA reduced, in a dose related fashion, the amplitude of the ERG b wave (up to 40%) in both scotopic and photopic conditions without significant changes in the implicit time. No effects were seen at the level of the a-wave. These results suggest that the D2 receptor is implicated in modulation of the b-wave, whereas the D1 receptor is involved in the modulation of the a-wave.

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EXPRESSSION OF METABOTROPIC GLUTAMATE RECEPTOR mRNAs IN ADULT RAT RETINA. E. Hayworth* and J. H. Brandstatter, Max-Planck-Institut für Hirnforschung, Neuonauten, Alt., Frankfurt, Germany.

Glutamate is the major neurotransmitter in the vertebrate retina and retina is a unique area of neurons and glia characterized by a series of ionotropic and metabotropic glutamate receptors which have been cloned and characterized. While the major glutamatergic neurotransmission in the retina seems to be mediated by ionotropic receptors, the extent of involvement of metabotropic receptors in retinal function is unclear. We have therefore investigated expression of patterns of six metabotropic glutamate receptor (mGluR) subtypes in situ hybridization, using digoxigenin-labeled cRNA probes and then exposed to anti-digoxigenin alkaline phosphatase and staining with substrate. mGluR1 is expressed in all cells in the ganglion cell layer (GCL) and by some cells with their somata in the innermost layer of the inner nuclear layer (INL), most likely amacrine cells. mGluR2: expressed by some cells in the GCL and by some amacrine cells in the INL. mGluR3: not expressed at detectable levels. mGluR4: expressed by cells in the GCL and the INL, similar pattern to that seen for mGluR1. mGluR5: expressed by cells with their somata in the outer third of the INL, possibly horizontal cells. mGluR6: expressed by cells with somata located in the outer half of the INL, consistent with the recent suggestion by Nakajima et al. (1993) that this receptor corresponds to the physiologically and pharmacologically defined APB-receptor that mediates synaptic input from photoreceptors to ON-bipolar cells. Taken together, these results suggest a considerably more widespread expression of the in retina than suggested from physiological experiments. We are currently extending this investigation by examining the expression of the recently cloned mGluR7 and improving the cellular resolution by in situ hybridization of dissociated cells. Supported by a HHF Foundation (HHF) and SFB 269/B4.

GONADOTROPIN OF ACETYLCHOLINESTERASE (AChE) IN THE DEVELOPING OPUSCULAR RETINA. Camarao, L.M.C.; Pariá, A.C. and J.H. Holok*. Depto. Histolgia & Embriologia (ICCH); 1Instituto de Biofisica Carlos Chagas Jr., Universidade Federal do Rio de Janeiro, 21949-900, BRASIL.

We have previously shown that the differentiation of choline containing neurons in the retina was detected around 15 postnatal days (P15). In this work, we follow the pattern of expression of AChE, the hydrolyzing enzyme for acetylcholine, during the development of the retina, applying the Hemagreen method (1985). The onset of AChE activity was seen in the P10 opossum, in cell bodies present in the distal layer of the retina (P15). This activity is detected before the emergence of the inner plexiform layer (IPL) and before the appearance of cholinergic neurons, much earlier than the onset of synthesis of acetylcholine in the IPL (P22). Between P25-P33, the histochemistry reaction becomes more intense, but is still restricted to cell bodies in the GCL. Only after P45 AChE positive bands in the IPL (strata 1, 3 and 4/5) and somata in the inner nuclear layer (INL) could be seen. In P50, when the retina was morphologically mature and the AChE-histochemistry was the same pattern, i.e., cell bodies in the GCL and INL, labeled bands in the IPL and OPL. Since AChE could be detected so early during opossum retinal development, a question arises whether AChE takes part on cellular proliferation and/or differentiation in the retina.

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GABA RAISES INTRACELLULAR CALCIUM IN NEURONS OF DEVELOPING RABBIT RETINA. B. Huang* D. Redburn Dept. Neurobiology and Anatomy, Univ. Texas-Houston Med. Sch., Houston, TX 77030.

Previous studies in this laboratory have shown that horizontal cells of the rabbit retina are transiently GABAergic during early post-natal development and that lesioning these horizontal cells or blocking GABA receptors with antagonists interrupts cone synaptogenesis. In order to determine the mechanism by which GABA might mediate these effects, we have used a Ca^2+ fluorescent assay to explore the effects of GABA on calcium flux in developing retinal neurons.

Cells from neonatal rabbit retina were freshly isolated and then loaded with fluo-3 AM. When challenged with 100 μM GABA, intracellular Ca^2+ increased in certain populations of retinal neurons. This GABA-induced Ca^2+ response began to appear in retinal cells of postnatal day 3 and was most prominent on day 5. Both picrotoxin and bicuculline blocked the response, indicating that GABA, receptors were involved. Based on these results, we propose that during postnatal retinal development GABA may exert its trophic action on retinal synaptogenesis through membrane depolarization and a rise in intracellular Ca^2+.

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CIRCADIAN RHYTHMS IN THE QUAIL RETINA: MODULATION BY LIGHT AND DOPAMINE

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The Japanese quail exhibits a circadian rhythm in retinal sensitivity. 1 Rhythmic shifting of rod-cone dominance with time of day appears to be intensity dependent. Circadian rhythms in dark-adapted eyes are readily detectable for photopic stimuli: rods dominate retinal sensitivity at night while cones dominate during the day. Suprisingly, for scotopic stimuli, rods dominate retinal sensitivity regardless of time of day, thus abolishing circadian rhythms of sensitivity.

How does the circadian clock shift rod-cone dominance during the day but not at night? We hypothesize that dopamine blocks rod signals to increase stimuli during the day and thereby shifts the retina to the cone-dominated state. Retinal dopamine levels are high during the day and low at night in constant dark making dopamine an obvious candidate for modulating retinal sensitivity. Dopamine agonists and antagonists alter the rhythms of retinal sensitivity, and light adaptation can disrupt the normal rod dominance at night. 1,2 Primary retinal neurons, light and dopamine are independent modulators of the ocular clock in the Japanese quail.

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The area 3b cortex of primates contains hand- and foot-like aggregates of neurons that are dominated by cutaneous inputs from the ulnar, radial, and median nerves to the hand. The ulnar nerve innervates about 43% of the hand surface, and normally provides dominant inputs to about 20% of the area 3b hand cortex. This study evaluated whether ulnar nerve dominance aggregates change in size over the first several minutes to hours after wrist level transection of the radial and median nerves. Acutely after injury, cortical aggregates of the ulnar nerve rapidly expanded from a normal mean area of about 4.9 mm^2 to a mean area of about 7.1 mm^2. Due to this change, ulnar inputs rapidly gain access to about 59% of the area 3b hand cortex, or about 1.5 times the normal cortical surface. These size changes are initiated within minutes after injury. The extent and spatial distribution of these changes provide an image of central substrates that were available for decompensation of ulnar nerve dominance aggregates at the time of injury. Previous evaluations of radial nerve dominance aggregates after acute transection of the ulnar and median nerves show that these aggregates rapidly gain access to a cortical space that is about 2 times the normal cortical space. Thus, dominance aggregates of different nerves undergo rapid size changes of variable extents. It is suggested that this variability is dependent on differences in the patterns of central connections that are rapidly impacted by the different injuries, and on differences in the patterns of central substrates that can be rapidly accessed by the uninjured nerve.

Supported by NS 27118S.

Reorganization of Movement Representations in Primary Motor Cortex of Adult Squirrel Monkeys Following Distal Forelimb Restriction. G.W. Bilimben*, E.J. Plautz, G.A. Gardner, B. Raiszadeh, and R.J. Nudo* Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77030.

The consequences of distal forelimb restriction on the functional topography of primary motor cortex (area 4) were examined in normal adult squirrel monkeys. Using intracortical microstimulation techniques, a detailed map of the movement representations of the hand, wrist, and arm contralateral to the preferred hand, and the hand of the nonpreferred hand, were derived (Nudo, et al., J. Neurosci., 12:918, 1992). After a post-operative recovery period the preferred forelimb was placed in a cast that effectively restricted movements of the hand and wrist. Movement maps of the distal forelimb representation were re-derived at 3 month intervals.

In each case, post-restriction maps revealed significant changes in movement representations. The movement show that within 6 months, finger representations decreased while wrist representations increased in total area extent. In contrast, the movement representations of control animals that did not undergo forelimb restriction remained relatively unchanged. These results demonstrate that distal forelimb movement representations are alterable through disuse, and that the changes associated with forelimb restriction are progressive over several months. Taken together with our previous findings that forelimb representations are alterable through increased use, these studies lend further support to the idea that movement representations in primary motor cortex are shaped by experiential factors related to use.

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The motor cortical maps in adult mammals are reported to reorganize following various manipulations including peripheral denervation (Donoghue et al, 1990), repetitive intracortical microstimulation (ICMS) (Nudo et al 1990a) and local disinhibition by intracortical baclofen infusion (Jacobs and Donoghue 1991).

These studies suggest that differential activities in competing regions within the primary motor cortex (MI) mediate the reorganization of motor representations. The present experiment examined this hypothesis by recording multiple unit activity (MUA) from vibrissal and other regions of MI before and after facial nerve transection.

Three to seven microelectrodes were placed at low threshold depths of the right MI in ketamine-anaesthetized rats. The same microelectrodes were used for ICMS and MUA recording. Multisite MUA, ICMS-evoked movements and electromyograms of left facial, neck and forelimb muscles were recorded before and after transection of the buccal and mandibular branches of the left facial nerve. The MUA at sites recording primary vibrissal movements was greatly reduced, compared with other sites, immediately after the transection and remained so up to several hours. This result suggests that the differential neural activities in the MI might play a role in MUA reorganization. The MUA reduction in vibrissal regions of MI may be due to a decrease in vibrissal (trigeminal) afferent tone resulting from the motor nerve transection.
507.5 CORRELATION DIMENSION AND LYAPUNOV EXPONENT CALCULATIONS FOR NEURAL ACTIVITY RECORDED IN PRIMATES DURING VOLUNTARY TWO-DIMENSIONAL HAND MOVEMENT WITH INSTRUCTED DEGREES OF FREEDOM

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In addition to linear methods, such as Fourier transforms, autocorrelations and crosscorrelations, nonlinear dynamical analysis methods were used to examine multichannel cortical activity in order to characterize the spatio-temporal dynamics of the cortical network during movement performance (Gall et al., 1991). We used the method of estimated local field potentials (LFP) proved to be nondirectional in a planar directional hand movement task using instructed delay, while units showed directional selectivity (Donoghue et al., 1994), this volume and extracellular unit activity were subjected to correlation dimension (CD) and largest Lyapunov exponent (LE) calculations using MTRACHOS and MTrLyAPV software developed by R. M. Rosenstein (Rosenstein et al., 1993; Physica D 65) as well as Chaos Data Analyzer distributed by the American Physics Institute. Both LFP and unit data turned out to be higher than those observed in the past. The high dimension correlation results obtained indicate that no simple set of equations can be derived to model the system. New methods, such as complexity calculations (Rapp et al., J. Neurosci, 1994) have been used to further investigate extracellular spikes and activity.

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507.7 THE POTENTIAL FUNCTIONAL ROLE OF REVERBERATING SYNAPSE CHAINS DEVELOPING IN RANDOMLY CONNECTED NEURAL NETWORKS

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Over the past few years, several types of synchronized neural activities have been observed in different cortical areas. This review gives rise to a theoretical debate on the potential role of such synchronizations for cortical function. Experimental results on the abundance of certain events and dependence of cortical phenomena on the spatio-temporal spike patterns in the prefrontal cortex of awake behaving monkey ( Abeles et al. 1993) support the hypothesis, that 'synaptic volley' propagate through the cortex in 'reverberating synapse chain' (RSC). Feedback networks with additional feedback connections.

To get insight into the possible functional role of such RSCs, we investigated their development in a model neural network (see also Brennerstock, 1991). An initially randomly connected network of excitatory and inhibitory neurons with poisson-distributed coupling strength was repeatedly stimulated by a single synchronized spike event, adjusted to activate only a small fraction of the neurons in the network. The global dynamics of the resulting activity spread through the network can be described analytically following Amoroso et al. (1970). Concomitantly, the synaptic connectivity changes gradually as a result of interaction with a 'competitive learning rule'. This development leads eventually to the formation of RSC. Moreover, interactions between different RSCs develop, depending on the spatio-temporal structure of the corresponding stimulus. The path through the network defined by the RSC, may be interpreted functionally as a trace of auto- and hetero-associations. Thus, the space-time composition of the RSCs and their interconnections provide a natural implementation of stimulus context memory.

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507.9 THE PROFILE OF VOLTAGE-DEPENDENT CONDUCTANCES IN NEOCORTICAL LAYER 1 NEURONS

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Layer I of the mammalian neocortex contains >90% GABAAergic interneurons and a very low neuronal density. Presumably because of its low density, it has proven to be extremely difficult to record from layer 1 cells. Consequently, nothing is known about the neurons' intrinsic firing properties or the complement of ion channels underlying these properties.

We studied membrane currents in acutely dissociated layer 1 neurons obtained from the rat layer by minimizing layer I from neocortical slices and subjecting the pieces to enzymatic digestion. Inward currents exhibited by layer I neurons included a fast-inactivating, low-threshold Ca2+-current, which, in some neurons was followed by a persistent K+-current. No evidence was found for a persistent Na+-current. TTX-resistant Na+-current, or low-threshold Ca2+-current. Outward currents included a delayed K+-current, an A-current, and a Ca2+-activated K+-current. Further characterization of the Ca2+-current included an inward rectification of TTX-sensitive Ca2+-current at negative potentials.

The profile in layer 1 cells differs from those reported previously in pyramidal or nonpyramidal neurons from lower layers. Unlike nonpyramidal cells, mature layer I cells express an A-current. Unlike pyramidal cells, layer I cells do not exhibit higher dimensional than results of previous relevant calculations in this particular complement of ion channels will be discussed.

Supported by grants from the ONR and NIH

507.10 PHYSIOLOGICAL SUBGROUPS OF NONPYRAMIDAL CELLS IN LAYER II/III OF RAT FRONTAL CORTEX Y. Kawaguchi1, Lab. for Neural Circuits, Bio-Mimetic Control Research Center, RIKEN, Saitama, Japan

Physiological and morphological properties of nonpyramidal cells in layer II/III of frontal cortex of young rats were studied in vitro by whole cell recording and intracellular staining of Nissl. Layer II/III nonpyramidal cells could be divided into four subgroups by their firing patterns in response to depolarizing pulses and patterns of dendritic and axonal arborizations. (1) Fast-spiking (FS) cells had shorter-duration action potentials and constant spike frequencies during an episode of discharges. FS cells had local or horizontal axonal arbors which did not enter layer I. FS cells were strongly activated by GABA-A and parvalbumin cells. (2) Late-spiking (LS) cells exhibited slowly-developing ramp depolarizations near threshold. LS cells were neurogliaform cells. (3) Low-threshold spike (LTS) cells had low-threshold spikes with larger amplitude and longer duration. The main axons of LTS cells ascended, and the collaterals entered into layer I. (4) Although the remaining cells (regular-spiking (RS) nonpyramidal cells) did not have clearly distinguishable electrophysiological properties, RS cells are tentatively classified into two types. (a) RS type 1 (RS-1) cells induced depolarizing fast notches with smaller amplitude by depolarization from hyperpolarized potentials. RS-1 cells had vertically elongated axonal fields, extending from layer I to V, sometimes to layer VI. This type contained bipolar cells. (b) RS type 2 (RS-2) cells extended axonal branches vertically, giving rise to layer I, RS-2 cells also contained a chandelier cell. These suggest that each subgroup may differentially contribute to the laminar and columnar circuitry of cortex.
570.11
PYRAMIDAL MOTOR EVOKED POTENTIALS EVOKED BY CORTEX STIMULATION IN RATS. Y.C. Park*, J.W. Chang, J.H. Kim and S.S. Chung, Dept. of Neurosurgery, Kyung Hee University Hospital, Seoul, Korea
Motor evoked potential (MEP) produced by cortical surface stimulation has recently evolved as a new clinical and experimental tool to study excitability of motor pathways. The original concept using MEPs was based on the assumption that synchronized neuronal discharges evoked by electrical stimulation of pyramidal neurons in the motor cortex were conducted along the corticospinal tracts in the spinal cord. It was, however, recently found that MEPs in the spinal cord were mainly produced by activation of reticular systems in the brain stems due to spread of stimulus current applied on the motor cortex, especially in non-patients with intact brain.

The purpose of this study was, therefore, to selectively monitor MEPs evoked by cortical areas in the rat. A small surface electrode with a diameter of 1.5 mm and another electrode protruded 1.5 mm from the surface electrode were used as an anode and cathode, respectively. The evoked responses were recorded on the spinal cord using bipolar electrodes. The potentials monitored were compared with their origin since the potentials were abolished when the pyramidal tract was selectively lesioned at the internal capsule. The intracord recording of the MEPs showed that the amplitude was highest at the ventralmost area of the dorsal column where the corticospinal tract was located. Latencies of these potentials were much longer than the latencies of the non-pyramidal MEPs. Amplitudes, latencies and wave forms of these potentials were studied following intravenous injection of various general anesthetics to examine the effect of these drugs on MEPS.

571.1
MULTIPLE MOVEMENTS MAY BE RELIABLY DIFFERENTIATED USING fmri, PROVIDING ORGANIZATIONAL PERSPECTIVES ON BRAIN FUNCTION. T.W. Kier*, A.A. Hertz, P. Jessard, T.P. Pons, B.J. Richmond. Lab. of Neuropsychology, INSERM, Bethesda MD, USA; Nordia, Copenhagen, Denmark; Lab. of Cardiac Energetics, NHLBI/NIH, Bethesda, MD, USA.
We have investigated the differentiability of activations in the human brain during several randomly-interleaved self-paced motor tasks, as revealed in functional magnetic resonance imaging (fMRI). We used a neural network as a distribution-free technique to classify the tasks on the basis of the fMRI signal. This approach directly estimates probabilities of multiple conditions from an fMRI picture (or any set in area x time). It does not make a priori assumptions about the distribution of the signal or the relative signal strength for different conditions. Discriminability is computed as the information content in the image about the classification set.

Different sets of fMRI-amplitude data from four motor tasks were analyzed. The analyses were carried out on 6 spatial scales, from individual voxels to the full picture. In some cases, the data were preprocessed using principal components. In general, information was found to be encoded across many different voxels. However some voxels were found to be completely independent of their neighbors. Hence spatial smoothing of fMRI data (by intentional smoothing approx.) is undesirable.

To determine whether the overall discriminability of a set of conditions was due only to the discriminability of a few of them, we repeated the analyses for subsets of the conditions and compared the information carried out about these subsets that conveyed about the entire condition set. In this way we found areas in the lower part of the frontal lobe where the information in the signal was larger for 4 conditions than for any subset of 2 conditions. Another area at the frontoparietal junction only permits differentiation between pairs of conditions. For these areas, there is no information in the pictures about added conditions. On the basis of these results, we conjecture that the first set of areas could be involved in planning movements and the second in specific movement components.

571.3
Neuromagnetic fields elicited from the left hemisphere of five healthy right-handed subjects were investigated under three different experimental conditions run in random order every 5-8 s: electrical stimulation of the right index finger (task S), voluntary movement of the right index finger (M), and "interference" condition consisting of voluntary movements triggering the S at the very beginning of the electromyogram of the active muscle. The activity of the sources corresponding to the main components of the SEFs and movement-related components was mapped and localized by means of a moving dipole model. The gating effect was measured by S/(M+S-M). In all the subjects recorded the gating effect was observed starting at approx. 30 ms after movement onset and lasting for the whole period of the ongoing movement. The site of the early gating effect (mostcentrally) was found to be more closely located than the later (mostly centroparietal) gating effect. The task SEFs were found to be larger (significantly after 40 ms) than the control SEFs elicited in a basal condition.

The results in terms of the gating, the mechanism (centrifugal and centripetal), and locus and selectivity of the somatosensory gating. The interesting cortical finding about larger task SEFs as compared to the control SEFs is interpreted in terms of specific attentional influences upon the components after 40 ms.
571.5
DOES THE CORTEX PROCESS POSTURAL ADJUSTMENTS ASSOCIATED WITH BALLISTIC MOVEMENTS? E. Palmer*, E. Cadopoli, P. Ashby. Playfair Neurosience Unit, Toronto Western Hospital, Toronto, Canada.

When seated subjects rapidly abduct one arm in response to a tone, EMG activity occurs in the deltoid of that arm, and, almost simultaneously, in the contralateral latisissimus dorsi. The contralateral activity is assumed to be an "associated postural adjustment". When subjects abducted the left arm rapidly, magnetic stimulation over the left motor cortex delayed the onset of the EMG burst in the right latissimus dorsi relative to the initial burst in the left deltoid. When subjects abducted the right arm rapidly, magnetic stimulation over the left motor cortex delayed the onset of the initial EMG burst in the right deltoid relative to the burst in the left latissimus dorsi. In each case, the delay was greatest when the stimulus was given just before the burst was expected to occur. The inhibition of voluntary movements by transcranial stimulation was not associated with a reduction in the excitability of spinal motorneurons. We postulate that both the focal ballistic movement and the associated postural adjustment are preprogrammed, held in memory until the "go" signal and then released through both motor cortices to spinal neurons. (Supported by MRC 6727)

571.6
MOTOR EVOKED POTENTIALS (MEPS) TO PAIRED TRANSCRANIAL MAGNETIC STIMULI SHOW DIFFERENCES DURING REST, ACTIVATION AND POST-EXERCISE FACILITATION. J. Sanfilippo, M. Halil, S. Grill and E.M. Wassermann. Human Motor Control Section, NINUS, Bethesda, MD 20892

A TMS pulse to the motor cortex at an intensity of 1 x threshold inhibits the MEP produced by a second pulse at intervals (ISIs) from about 40 to 150 ms (Valle-Inclan et al., 1993). But maximal inhibition of the MEP occurs when ISIs are in the range of 15-20 ms after 10 sec periods of 20% maximal voluntary contraction. In both subjects activation caused a large increase in the amplitude of the first MEP and a progressive decrease of inhibition at ISIs greater than about 100 ms which was not seen at rest. Post-exercise facilitation caused a significantly smaller increase in the amplitude of the first MEP in both subjects, but complete reversal of the inhibition of the second MEP in one subject and frank facilitation of the second MEP in the other. Post-exercise facilitation has a weaker facilitatory effect on the first MEP, but it can cause facilitation or a greater reduction in inhibition after the MEP than voluntary activation.

571.7
RESPONSES TO TRANSCRANIAL MAGNETIC STIMULATION DURING FATIGUING MAXIMUM VOLUNTARY CONTRACTION W.B. McKay*, S.M. Tael, A.M. Sherwood, M.R. Dimitrijevic, Div Research and Neurology, Human Neurology, Baylor College of Medicine, Houston, Texas

Sustained maximum voluntary contraction (MVC) is a widely used paradigm for the study of fatigue mechanisms. Cortical motor output was measured by surface recorded motor evoked potentials (MEPs) resulting from transcranial magnetic stimulation (TMS) of the motor cortex. TMS at 80% of maximum (2.0 Joules) was delivered over the Cz scalp EEG location. MEPs and M-waves from supramaximal nerve stimulation (SNS) were recorded at 15 second intervals before and during MVC of ankle dorsiflexors in six healthy human subjects. The subjects were asked to maintain MVC of the right ankle dorsiflexors until the force developed decreased to 50% of its initial value. During ankle MVC, MEP activity, M-waves, and muscle activity were monitored. The MEP amplitude to right and left quadriceps, hamstrings, anterior tibia and triceps surae muscles increased compared to pre-fatigue conditions. The changes in the largest increase occurred in the exercised anterior tibial muscle (p < 0.05). Also, MEP amplitudes in the contracted anterior tibial muscle were maintained while its voluntary activity amplitude and median EMG frequency and force of contraction decreased. Anterior tibial muscle M-wave amplitudes did not change but twitch forces decreased during the contraction. The results show that motor cortex output for single TMS delivery does not decrease while voluntary force generation and EMG decrease during sustained MVC.

571.8

Controversy in human mapping studies of unilateral movements relates to bilaterality, quantitation, and spatial extent of the activations. We used quantitative measurements of regional cerebral blood flow (rCBF) with [15O]-butanol and positron emission tomography for mapping of simple finger movements using a computerized task, and [15O]-water for mapping of cortical activity related to the number of words produced for a given word category. rCBF at rest was measured using a time-locked approach. Similar results were obtained at rest and during voluntary index flexion movements. A preliminary analysis of a large number of words was presented at the symposium. Significant increases in rCBF were seen in the left premotor cortex (650 ml) and motor cortex (260 ml) in both hemispheres, but larger increases were seen in the left hemisphere. Increases were noted in the supplementary motor cortex, both dorsal and ventral premotor cortex, and the posterior parietal cortex. No increases were observed in the cerebellum or the thalamus. The increases in rCBF were significantly larger in the left hemisphere. The increases were influenced by the number of words produced, with larger increases as the number of words increased. The results indicate that the human motor system is bilaterally activated for a simple finger movement task, with greater involvement of the left hemisphere. The increases were seen in the primary motor cortex, supplementary motor cortex, and the posterior parietal cortex. No increases were observed in the cerebellum or the thalamus. The increases in rCBF were significantly larger in the left hemisphere. The increases were influenced by the number of words produced, with larger increases as the number of words increased. The results indicate that the human motor system is bilaterally activated for a simple finger movement task, with greater involvement of the left hemisphere.

571.9
VIBRATORY, MOTOR AND TRANSCRANIAL MAGNETIC STIMULATION IN HUMANS PRODUCE A LOCALIZED CHANGE IN CEREBRAL OXYGENATION DEMONSTRATED WITH NEAR INFRARED SPECTROSCOPY. H. Chou, B. Brantzen, R. Meyer, GID Biometrics, U. Dinagd, A. Vingerling, Dept. of Neurology, Humboldt-University, Berlin, and Ludwig-Maximilians-University, Munich FRG

We examined the potential of Near Infrared Spectroscopy (NIRS) to detect localized changes in concentrations of oxygenated and deoxygenated hemoglobin ([Oxy-Hb],[deoxy-Hb]), [total-Hb]=[Oxy-Hb]+[deoxy-Hb]) with a non-invasive near infrared spectroscopy monitor (NIRS-500, Hamamatsu). The following tests were studied: (1) a sequential motor task, (2) magnetic stimulation, (3) vibratory stimulation and (4) electrical median nerve stimulation. Optodes were positioned according to a modified 10/20 system. In 3 experiments, optode position was assessed by T1-weighted-MRI. (1) In 20 subjects a finger opposition task ipsi- and contra-lateral to optode position over left primary motor cortex was repea-tedly performed in a time-locked fashion to movement onset. A clear rise of [Oxy-Hb] and [total-Hb] was accompanied by a decrease of [deoxy-Hb] detected exhibiting a pro-nounced contralateral predominance. (2) In 5 subjects transcranial magnetic stimulation was applied over the mid-point between the optodes resulting in a contralateral finger movement. Voluntar-y movement of the same finger was performed in the same experiment. Magnetic stimulation-induced movement resulted in smaller changes of NIRS-parameters. (3) A localized response to a vibratory stimulus was elicited over primary somatosensory cortex, whereas (4) electrical stimulation of median nerve did not show a clear response. The experiments show that NIRS is able to detect rather small changes of local cerebral oxygenation, though optode positioning remains a problem. Supported by the DFG VI 387/1-
571.11 SPATIAL AND TEMPORAL DISTURBANCES IN PATIENTS WITH PARALYTIC LESIONS WITH AND WITHOUT IDIOMETOR APRAXIA. R. Hether, F. Weiss, S. Neumann, C. Dode, H. Kuhlmann and H. J. Freund. Department of Neurology, Univ. of Dusseldorf, 4002 Dusseldorf, Germany. Lesions of the parietal cortex lead to severe motor disorders, known as apraxia for left- and hemispheric for right-hemispheric lesions. In a retrospective study, 6 patients with parietal lesions (4 with and 2 without idiometor apraxia) and 6 patients with right parietal lesions were tested for spatial and temporal errors and the presence of a neglect component. Movement trajectories were recorded by a Selspot IIP system and a video camera. During the first set of experiments patients had to produce horizontal, vertical, and circular arm movement components in their personal space, each consisting of 10 movement sequences (SMS) were composed. These SMS had to be produced after reading aloud the instructions ('reading'), after verbal instructions ('instruction'), and after the experimenter had demonstrated the movement ('demonstration'). The 5 SMS and the 1 model of instruction were randomized for each side. For all patients the same sequence was used. In patients with left parietal lesions, significantly more spatial (direction reversions) and sequencing errors (wrong order of movement components) of the movement trajectory were observed in comparison to the right parietal lesioned patients. More omissions or repetitions of movement components revealed aspects of motor neglect or perseveration errors. In the second set of experiments, the patients had to grasp a 10 cm long stylus (lying horizontally in front of them) after eye opening without previous visual feedback. Whether hand inversion (HI) or hand elevation (HE) is used for grasping depends on the angle of the stylus in relation to the sagittal plane resulting in a (HIHE) probability curve. In normals, the HIHE-probability varied within a small range of 20 degrees only. In both parietal groups, especially in the left hemispheric patients with apraxia, this angle range was significantly enlarged to more than 100 degrees in comparison to the controls.

We conclude, that the right parietal cortex is mainly involved in the decision "what to do" and the left parietal cortex in the problem "how to do" a motor task. Thus, normal motor behavior affords an intensive interaction between both parietal cortices.


Our recent studies in the cat (Cullen et al. 1991) have demonstrated that IBM discharges are best correlated, not with the metrics of the movement of the eye in the head but with the movement of the visual axis in space (gaze) during rapid orienting coordinated eye and head movements. To further address whether cal and monkey IBNs carry gaze or eye related signals, we carried out a dynamic analysis of IBM discharge. The activity of IBNs reflects an up-swing segment which encodes a non-linear representation of motor error (Van Gelsenberg et al. 1981) was first tested. For our neurons, discharges head-fast or head-fixed were significantly less correlated with a quasi-linear representation of eye or gaze motor-error, than by simple downstream dynamic models which incorporated eye or gaze velocity and a firing rate bias term. For downstream models, we found that gaze velocity was better at predicting IBM activity than eye velocity. The estimated firing rate based on a model using gaze velocity produced significantly better fits of the actual firing patterns, which was based on a quasi-linear model (> 30% less RMS). Adding a bias term to the gaze and eye velocity models improved the fit of both models. Interestingly, the value of the bias estimated for the gaze model was comparable to that which we determined for head-fixed models, while the estimated value for the eye model was significantly larger. Since the bias term caused the physiological "resting discharge" of the IBM, it seems important to determine why this bias is not present, and why both of these models perform so well. The firing rate of the neurons studied was correlated with the metrics of the eye and/or gaze movements.

572.2 INTERACTION BETWEEN VERTICAL EYE POSITION-RELATED NEURONS IN AND AROUND THE INTERSTITIAL NUCLEUS OF CAJAL ON BOTH SIDES. Y. Iwamoto*, S. Chimoto, E. Nambo, K. Tohvida. Dept. Physiology, Inst. Basic Med. Sci., Univ. of Tsukuba, Tsukuba, 305 Japan. We have recently shown that many neurons in and around the interstitial nucleus of Cajal (INC) that project contralaterally carry vertical eye position signals. In the present study, we recorded activity of vertical eye position-related INC neurons and examined electrophysiologically their aural course and synaptic nature in the alert cat. For 11 downward-on and 5 upward-on neurons, depth profiles of threshold and latency for antidromic activation were studied by tracking in and around the contralateral (c-INC). These neurons were activated from not only c-INC but also a region 1-2 mm dorsal to c-INC. The latency became longer as the stimulation site moved ventrally, showing that the stem axon coursed ventrally toward c-INC. Double or triple low-threshold peaks were observed in 4 downward-on and 2 upward-on neurons when stimuli were applied to c-INC, indicating the existence of branches. Stimulation of the midline region suggested that these neurons project to the commissural and/or efferent pontine commissure. Stimulation of c-INC and the more dorsal region inhibited spike generation of 7 downward-on and 2 upward-on neurons that were not laterally inhibited. The range of connection was from 0.7 to 1.4 msec. Effective stimulation sites seemed to be distributed along the course of fibers from PC. Results suggest that these non-contralaterally projecting neurons project to commissural INC neurons on the contralateral side, most likely from those having an opposite orientation.

572.3 AN EGG-BASED BRAIN-COMPUTER INTERFACE: USE BY INDIVIDUALS WITH ALS. D. J. McFarland*, L. McCane, T. Vaughan, and J. R. Wolpaw. Wadsworth Labs, NY State Dept Health and SUNY, Albany, NY 12201. Humans can learn to control the amplitude of EEG activity recorded over sensorimotor cortex (e.g., the 8-12 Hz mu rhythm) and use it to move a cursor to a target on a video screen (Electroenceph clin Neurophysiol 78:252-259, 1991 and in press). Brain-computer interface (BCI) systems link the synchronization of motor imagery to a new communication channel for those with severe motor disabilities. We are using high-resolution EEG frequency and topographic analysis to improve this technology and are investigating its applicability to individuals with specific motor disabilities.

After initial evaluation of the EEG and its relationships to sensorimotor activity, subjects learn to control cursor movement through 24 channels of EEG are stored for offline analysis aimed at improving the algorithm that converts EEG activity into cursor movement.

In initial studies, individuals with early-stage amyotrophic lateral sclerosis (ALS) were able to learn to control cursor movement. If this control persists as the disease progresses, EEG-based communication could prove of considerable value in the later stages, when useful voluntary movement is largely or totally gone. (Supported by the National Center for Medical Rehabilitation Research (NIH grant HD 30146).)
572.5


The movement field properties and the d-charge dynamics of 206 MRF neurons located lateral to the oculomotor complex have been studied in 2 awake behaving monkeys performing visually guided saccades. At least two major clusters of neurons can be distinguished. One group, the long lead burst neurons, has a build-up of activity which begins 150 msec before saccades, an accelerated burst just before the saccade, this burst declines during the saccade, and the tail of activity may continue for 50 msec after saccade end. The horizontal saccade vector for these cells is primarily contraversive with a vertical component. The movement fields for these cells are large and open-ended. These cells are located in the more ventral and caudal portions of the MRF. A second group of cells is distinguished by a tightly coupled burst of activity which starts 20-30 msec before, peaks just prior to, and declines sharply during visually guided saccades with little post saccadic activity (i.e. clipped cells). These tightly coupled cells can have either large (open-ended) or small (closed) movement fields. While many of these cells have omnidirectional discharges, the optimal saccade vector for these cells is again contraversal with a pronounced vertical component. These cells are located in the more dorsal and rostral portions of the MRF. The correlation of cell type with histologic location suggests at least two physiologically distinct regions of the MRF.

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572.6

EFFECTS OF REVERSIBLE LESIONS OF THE CENTRAL MESENCEPHALIC RETICULAR FORMATION (cMRF) ON PRIMATE SACCADIC EYE MOVEMENTS. D. M. Waitzman* and V. L. Slisak, Newington VAMC, 555 Willard Avenue, Newington, CT 06111.

Recent studies suggest that the cMRF participates in the generation of saccades, but its exact role remains unclear (Waitzman, Soc. Neurosci. Abstr., 1992). Visually guided saccadic eye movements of one awake rhesus monkey were recorded using a scleral search coil before and after the injection of 2% lidocaine (1 - 5 µl) and muscimol (1 - 3 µg) into the cMRF. Fifteen injections of lidocaine, 5 injections of muscimol, and 4 injections of saline were placed at a series of sites (containing saccade associated, multi-unit activity cells) which were at 1 mm increments along the rostral-caudal axis, 2 mm lateral to the oculomotor complex. Within 2 minutes of injection of either lidocaine or muscimol, contraversive horizontal and vertical saccades were hypometric, while vertical movements were hypometric. The primary position of gaze was shifted 3º down and to the ipsilateral side. The trajectories of vertical and oblique saccades were markedly curved toward the ipsilateral side. Injections 2 mm above or below the cMRF produced no effects on saccades. The effects on saccade trajectory suggest that the cMRF participates in the descending control of the horizontal component of movement, while changes in saccadic accuracy suggest the cMRF participates in the feedback control of saccade amplitude. The shift in the primary position of the eyes in the direction opposite to that of cMRF neurons is used in the maintenance of the primary position of the eyes. Supported by NIH Research Grant EY 09481 and a RAG Grant from the Office of Medical Research, Dept. of Veterans Affairs.

572.7

THE TIMING OF SACCADIC-RELATED RESPONSES OF BRAINSTEM OMNIPAUSE NEURONS IN CAT. M. Parducz* and D. Guirao, Montreal Neurological Institute and McGill Univ., Montreal H3A 2B4, Canada.

Recent evidence (Parducz and Guirao 1994) suggests that omnipause neurons (OPNs) in the superior colliculus project onto brainstem omnipause neurons (OPNs). Both cell types pause for eye saccades in monkey and gaze saccades in cat and it is of interest to determine whether they share other discharge properties. We recorded OPNs during visually-guided horizontal gaze shifts in the head-fixed and head-free cut and quantitatively analysed the temporal relation of their discharge relative to parameters of eye or gaze saccades. L. Cat OPNs ceased firing, on average, 25ms before saccade start. They resumed firing, on average, 6sec before saccade end. Some cells were reactivated after saccade end. Consequently, as already reported, the saccade duration was well correlated with saccade duration (r=0.9) but the slope of this relation was always less than one - a similar relation holds for monkey OPNs (Murao and Wurtz 1993). The slope increased linearly with the associated correlation coefficient, indicating that OPNs whose pauses are temporally well correlated to saccades have equal onset and end times. 2. Monkey OPNs are reactivated sooner for contraversive saccades compared to ipsiversive ones. The timing of the majority of our cat OPNs depended on saccade direction; two-thirds of the cells had significant left-right differences in pause end time. 3. Pause end time was twice as variable as pause onset time; the inverse has been observed for monkey OPNs. 4. OPN tonic discharge rate influenced pause timing: the higher a neuron’s firing rate, the closer to saccade start it stopped discharging. 5. Pause end time did not depend on any saccade parameters. On average, OPNs were reactivated at either ~16deg of gaze error or ~100m/s. In 1 and 2, OPNs and FPs have similar discharge characteristics. This suggests that OPNs are segregated into two lateralized populations of cells each receiving inputs from consular OPNs. However, the timing characteristics of OPNs are also clearly influenced by extra-FP inputs.

572.8


Botulinum neurotoxin A (BoNT) is an useful tool for the study of motoneuron-muscle interactions, since it produces a functional disconnection by blocking the release of acetylcholine at the neuromuscular junction. Are there any retrograde effects on the discharge characteristics of motoneurons when their target muscle is paralyzed with BoNT? To address this question, adult cats were prepared for the chronic recording of eye movements, lateral rectus muscle potentials and abducens motoneuron electrical activity. After control recordings, BoNT was injected into the lateral rectus muscle at either 3 or 0.3 mg/kg. Both doses produced in a few hours the paralysis of abducting eye movements that paralleled the disappearance of muscle field potentials induced by the electrical stimulation of the Vth nerve. The time course of these changes was shorter for the high BoNT dose and the recovery lasted longer. On the contrary, the high dose produced significant alterations in the discharge characteristics of abducens motoneurons. Thus, motoneurons lost their typical tonic-burst firing that accompanies eye fixations and saccades. Instead they showed a sustained low firing rate (15-50 Hz/pulse) and barely existed or cut-off in form that they were firing for the off-direction. Firing modulation for both spontaneous and vestibularly-induced eye movements was extremely weak yielding low sensitivities to both eye position and velocity. The signalizing deficits of motoneurons for off-directed eye movements were deeper than those for the on-direction. Accordingly, the scatterplots of firing rate vs eye position or velocity showed that two rather than one regression line fitted better the data and corresponded to the data partition into on- and off-directions of movement. The low dose of BoNT produced only subtle changes in the firing of some motoneurons despite total muscle paralysis. These data suggest that the firing alterations found result of a direct action of BoNT on motoneurons and not due to the target disconnection.

The synaptically focused population of abucescent interneuronal neurons with medial rectus (MR) motorneurons in the cat oculomotor nucleus have been examined by light and electron microscopy following injections of biocytin into the abucescent and vestibular nuclei, respectively. Both AB and IN synaptic terminals had a spherical shape, were small and uniformly distributed. About 1400 synaptic contacts were made with 6 motoneurones. Single biocytin filled terminals were followed extensively through the CNS, and made single synaptic contacts with distal dendrites, whereas most AB synaptic endings contacted proximal dendrites (98%) or soma (2%). IN synaptic endings were associated with either a postsynaptic density at sites of synaptic contact or axo-somatic synapses were in close apposition of the axon hillock. The abucescent pathway might function as a means of counteracting the effects of other inputs that are related to conjugate horizontal movements. Supported by USPHS Research Grant EY02191.

TRANSNEURAL TRANSPORT OF BIOCYTIN THROUGH ABUCESCENT MOTONEURONES OUTLINES THE INNERVIAL AND VESTIBULAR PATHWAYS IN TELEROST FISH. B. Berger, L. Marchi and P. Labbe. Dept. of Physiological and Biological Sciences, Univ. of Nantes, Inst. of Pharmacology, Nantes, France.

Selective injection of biocytin into the telerostral CNS of a diversity group of teleosts reveals retinal and cerebellar subgroups of abucescent motorneurons with intensely labeled dendrites and terminals in the extreme mediobasal extent of the hindbrain. Labeled immediately doro-medial to each sub-group and in about a 1.2 ratio are interneuronal neurons whose axons ascend dorsally in the medulla, pass cranially through the pons, and reach the midbrain, and then project rostrally in the diencephalic tract of the contra-MLF where they terminate onto other and possibly other motorneurons. In those species with rhythmiically conjugate horizontal scanning eye movements (e.g. goldfish, catfish vs. sculpin, sunfish), the bipartite oculomotor/nucleus interneuronal somata position is observed medially in the hindbrain and this location appears to be independent of either optic axis or location of the eye in the orbit. However, even when the eyes are horizontal in the midline, terminals are predominantly, but not exclusively, adjacent to the vestibular nucleus (e.g. midlenspman), the medially directed dendrites not only reach but also cross the midline. In all cases, labeled dendrites from each motoneuronal population with those of the adjacent interneuronal populations suggesting a similar embryonic origin of all neurones in rhombomeres 5 and 6. Transneural biocytin transport also marks the pathway and location of contralateral vestibular neurones in the descending oculovestibular nucleus included occasionally the axons of vestibular neurones that project to medial rectus motoneurons. Electron microscopy of the oculomotor and abucescent motor nucleus in goldfish reveals numerous axonometric and axodendritic synaptic endings showing membrane specializations characterizing chemical and gap junctions. Therefore, the extensive electrotropic coupling and transneural transport of biocytin prevalent in the lateral eye, the oculomotor and in other CNS regions would fulfill the essential demands of these neuronal subpopulations. Supported by USPHS Grant EY06968.

SIMULATION OF MOTOR UNIT RECRUITMENT IN EXTRAOCULAR MUSCLE. Paul Deutet*, Dept. of Psychology, University of Sheffield, Sheffield S10 2TN, England.

Understanding the recruitment pattern of extracellular motor units is important for constructing distributed models of oculomotor control. Recruitment in the abucescent nucleus was simulated using the adaptive model of motor unit recruitment, the firing rates of oculomotor neurones (e.g. Robinson and Keller, Bibl. Ophth. 82: 7, 1972), and the total active force developed in the lateral rectus muscle (e.g. Miller and Robinson, Comp. Biomed. Res. 17: 43S, 1984). The purpose was to determine the distribution of motor unit strengths that would fit both sets of data over the oculomotor range. The lateral rectus was represented as 100 motor units, initially of identical strength. For a given position of the eye, the force developed by each unit was calculated, and the sum of unit forces with the active force in the entire muscle as measured experimentally. The properties of the active units were then adjusted to reduce the size of any resultant error, in a manner related to gradient descent methods required to train the model. The simulated eye muscle was trained in the fitness for a series of eye positions drawn at random from the oculomotor range until performance stabilized. Flows of motor unit strength against oculomotor neurone threshold revealed an U-shaped pattern, with the strongest units being recruited at both extremes of the oculomotor range, and the weakest units recruited in the middle. The pattern remained qualitatively stable over a range of assumptions about the distribution of oculomotorneurone populations, and the relation between motorneurone firing rate and unit force. One part of the U-shaped pattern is similar to that observed in spinal motor neurones, where stronger units tend to have higher recruitment thresholds, whereas the other part is consistent with evidence from single motor-unit stimulation studies (Wall and Goldberg, Brain Res. 587: 291, 1992) suggesting that in eye muscles the strongest units are recruited first. The pattern as a whole may reflect the functional need for precise control of eye position in the middle of the oculomotor range. support of the grant USPHS Grant EY07446.


The performance of anti-saccades (AS) - saccades directed opposite to a visual target - entails the inhibition of reflexive saccade and the coordinate computation of a goal specified by instruction, i.e. not pointed to the oculo-motor representation of eye position seem mismatched. As a result, 2 flashes at the same retinal localization appear to be at different places. Could this illusory spatial mislocation improve the temporal discrimination of 2 stimuli flashed in rapid succession? Horizontal eye movements were recorded with an Ober eye-observer scanner in 2 human subjects, head fixed in the dark. Subjects were instructed to make a saccade to a 10 ms light (on left), 10 ms rightward from the point of fixation. During the latency period, another 4 ms light spot was flashed twice at 12° to the right of the point of fixation. Flash separation varied from 10 to 100 ms on the same lens the same time. When the same location localization appear to be at different places. The frequency of reporting errors as for eye positions was that of the saccadic onset. The frequency of reporting two flashes at different locations increased in parallel. We suggest that the improvement in temporal discrimination of 2 stimuli flashed in rapid succession of the eye position signal on visual input. (Supported by USHHS grant T32 GM038735 and USPHS grant EY058797).

BILATERAL PROJECTION OF SINGLE MOTOR NEURONS INVOLVED IN VISUOMOTOR CONTROL, A DOUBLE LABELLING STUDY IN THE RAT. P.A. Stringer (SFON: Brain Research Association). Department of Psychology, University of Central Lancaster, Preston PR1 2HE, UK.

Preliminary retrograde double labelling studies have identified oculomotor nucleus motoneurons projecting bilateral axonal collaterals through the retinotopical fields. This study was designed to combine retrograde / anterograde tracers in delineating these projections. Twenty male Wistar rats (230 - 330 g w.) were anesthetized with trinemethanol, and individual extracellular muslces, bilaterally, were injected (0.05 - 0.2 pL) through 50 μm tip diameter glass micropipettes connected to a Linear Flourograph (GF, Gil, Fast Blue (FB), PHA-I, BioIoylated Dextran Amine (BDA) or choleraotoxin - horsenized peroxidase (CTB). Survival times ranged from 48-96 hours, and following perfusion 80 μm sections were appropriately processed. Tryramethenidine diaminobenzidine and Vector VIP were used as chromogens with PHA-I, BDA and CTB. Fluorescent tracers were viewed with a Leica Discope. These studies showed only occasional bilateral innervation of extracellular muslces. A more common configuration would be, for example, for a ventromedially and rostrally located oculomotor neuron, retrogradely labelled only injection to the positions in the left inferior rectus, to also send an axon collateral to terminate in association the contralateral third nerve fascicles retrogradely labelled in turn following an injection of tracer to the right inferior rectus muscle. The results demonstrate that the anatomical bases of muscle linkages carried out at the simplest level of individual neurones. A primitive system overlap and increased in sophistication by the complex interconnections of premotor afferents. thursday am

OCCULOMOTOR: PERFORMANCE AND MODELING OF SACCADES
573.3


How the brain regulates saccadic initiation is not known. For useful neural correlates to be identified, novel behavioral methods are needed that systematically manipulate saccadic production. To manipulate the initiation of saccades, we implemented a countermarching paradigm (Lappe and Erkink, 1966, J Exp Psych, 72:805) in two rhesus monkeys. After fixation of a central spot, a target appeared at 1 of 2 locations. At the same time the fixation spot disappeared, signalling the monkey to generate a saccade to the target. On 25% of the trials the fixation spot reappeared after a delay, signalling the monkey to withhold the saccade. The stop-signal delay ranged from 25-225 ms. With short stop-signal delays monkeys withheld saccades to the target. On longer stop-signal delays monkeys increasingly failed to withhold the saccade. We observed a few trials in which monkeys generated a saccade failing short of the target, and on those trials a return saccade was usually made. The probability of saccade generation as a function of stop-signal delay increased monotonically with stop-signal delay in a sigmoid fashion (best fit logistic equation, r^2 = 0.97). The hypothesis that generation of the saccade is determined by a race between a go and a stop process provides a means of analyzing this inhibition function to estimate the covert latency of response to the stop signal (Logan and Cowan, 1984, Psych Rev, 91:295). For each stop-signal delay the stop-signal response time was estimated as the point on the cumulative distribution of go trial saccade latencies corresponding to the proportion of stop-trial results in which a saccade was generated for that stop-signal delay. Stop-signal response time increased with stop-signal delay from 140 ms at a 25 ms delay to 30 ms at a 225 ms delay. The average stop-signal reaction time for both monkeys was 80 ms. This countermarching paradigm will be useful to study the neural mechanisms that regulate saccade initiation. (Supported by ROI:EY08890 and T32:EY07135)

573.5


Saccades made to a visual target can have a bimodal latency distribution, the first mode of which is termed "express." If more than one target is presented, express saccades are suppressed (Schiller et al. 1987). It has also been reported that saccades occurring during the simultaneous presentation of two targets can be "averaging" and terminate between the targets. In this study, we examined the effect of averaging saccades and exhibit bimodal latency distributions. Two rhesus monkeys with implanted scleral search coils were used. At the start of a trial, a fixation spot was presented. After a randomized amount of time, this spot was extinguished and either one (80% of trials) or two (20%) targets were turned on at 6 deg. The two target condition, separations varied from 0.5 to 5.5 deg of arc. Saccadic latencies and their endpoints in space were analyzed. Whenever a distinct saccade population appeared, these saccades had bimodal latencies. As the proportion of the paired target was increased, the probability of averaging saccades declined and saccadic accuracy improved. Also, as saccades became less "averaging" and more "target directed", the express mode was markedly reduced. This study shows that express saccades can be made when two nearby targets are presented simultaneously, but they tend to land at averaged positions where there is no actual visual stimulus.

573.7


The existence of an express (E) and a regular (R) mode in distributions of saccadic reaction times (SRT) is now widely accepted fact (review, Fischer & Weber, Biol. Brain Sci., 16, 1993). The E-mode is often observed in the gap paradigm, where the offset of an attention attractor precedes target onset. These results led to the notion of "engaged" and "disengaged" attention. Without induced disengaged attention, two modes lead to a reduction of mean regular SRTs (Sadow, J Opt Soc Am., 57, 1967).

We have been interested in the relationship of SRTs to states of attention into a quantitative model. We assume that: (1) The attentional state can be mapped onto one continuous variable, a(t). (2) Aside from conduction delays, the total SRT is determined by a "processing time" which is mapped onto the relaxation of a(t), governed by stochastic dynamics of the form a + f(a) + e(t), down to a threshold. (3) e(t) is a Gaussian white-noise. (2) Two behavioural relevant "signals" act parametrically onto the attentional system: a 'warning' and a "go-signal." Without these signals the dynamics is bistable, reflecting the states of engaged/disengaged attention. To make the system switches spontaneously between the two states, so that they are visited with a certain probability. During the gap interval the system is still bistable, but now the disengaged state is more stable than the engaged state. Thus, the probability of being disengaged increases with time after the warm-up event. The go-signal duration. During the delay monophasic function x(t) crosses the threshold after a time which depends on the state at go signal onset. Simulations show the behavior of gap paradigms, including skewed SRT distributions and the dichotomy of E- and R-saccades as well as the reduction of regular SRTs with increasing gap interval. We acknowledge support from the European Communities, grant no. ERB4001CT920105.

573.8

EYE SACCADES GUIDED BY VISUAL, TACTILE AND PROPRIOCEPTIVE STIMULI IN MAN. O. Blanke, O.-J. Gröther and W.O. Guldin*, Department of Physiology, Freie Universität, 14195 Berlin, Germany.

Horizontal visual saccades (binocular stimulation with light-emitting diodes, distance up to ± 40 degrees horizontal eccentricity) were used to calibrate eye movements recording using a reflective infrared method). In addition to "visual" saccades the subjects performed saccades in the dark directed to the left and right index fingers positioned at a 90° or 180° angle (1970.7-1 per sec). The subjects were also asked without tactile stimulation to look at the index fingers (same position as in the preceding paradigm), whereby a short auditory kick determined the alternation frequency.

At an alternation rate of 0.7-4 per sec the "tactile" and "proprioceptive" saccades were significantly larger than the visually guided saccades in all but one subject, i.e. there was a considerable overshoot, indicating that the difference in the controlling eye position in the dark were operating at a gain below 1. This "error" in saccade amplitude increased with crossed hands. When visual saccades were suddenly changed to tactile or proprioceptive saccades, the increase in saccade amplitude occurred gradually with a time constant between 3 and 5 sec. The same was true when saccades were guided 20 times by two alternating diodes after an initial memory. (Supported in part by a grant from the Bundesministerium für Wissenschaft und Technologie, Germany).

574.1

INFLUENCE OF FAST SACCADE GAIN ADAPTATION AND FATIQUE ON SACCADE METRICS IN THE MONKEY. A. Straube, S. Ulster, P. R. Robinson, and A. F. Poach*, Neurologische Klinik, Ludwig-Maximillian University, Munich, Germany, and Department of Biology and Regional Primate Research Center, Univ. of Washington, Seattle, WA 98195, USA.

To provide accurate sacades, the oculomotor system must be able to compensate for changes in its neural elements. Such changes can be simulated by altering saccade gain either temporally decreased or increased the gain of visually-guided saccades by electronically detecting a targeting saccade and then causing the target to step either forward or backward by ±30° on these trials, this paradigm caused either a decrease or increase of saccadic gain to the initial target step by an average of 0.28 and 0.24 to 30% step, respectively in 3 monkeys. The adaptation in vertical or horizontal direction. The time constant for the readaptation towards the normal gain was always smaller than the time constant of the primary adaptation. For 30% backadaptation, the gain decrease was still 18% after 20 hours in the dark.

Associated with the gain change, however, there was also a change in saccade metrics. For both forward and backward adaptation, saccades were slower with longer durations. The percentage decrease in peak velocity was larger for larger saccades. It also varied from monkey to monkey and became less if sacades were adapted with a background. Saccade slowing could not be explained solely by fatigue because the same number of visually-guided sacades without a artificial target step caused smaller changes in duration and peak velocity.

Supported by grants from the Heisenberg Foundation and by NIH grants EY 00745 & EY 0799.

Translation of the stimulus induced rapid adaptation of horizontal saccade amplitude by contrast, rotation of the visual field did not affect a significant adaptation in torsion. Thus, our results do not support the hypothesis that Listing's law serves a visual purpose.

This work was supported by NWO and ESRRIT II Munich 6615.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
573.9 DYNAMICAL PROPERTIES OF AUDITORY EVOKED SACCADES M.A. Freas and A.J. van Opstal* Department of Medical Physics and Biophysics, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

We measured saccadic eye movements of human subjects towards visual and auditory targets through the oculomotor range. It was noted that auditory-evoked saccades had trajectories and velocity profiles that were less stereotyped than visually-evoked saccades. This often resulted in curved trajectories for saccades driven towards auditory targets in oblique directions. Close inspection of the velocity profiles showed that the main sequence properties of the horizontal and vertical components of auditory-evoked saccades seem to be at least in part, due to saccadic fixation. There is also evidence that the fact that the auditory system derives azimuth and elevation of a sound from different neural pathways. Therefore, convergence of these pathways in a late stage of the oculomotor pathway may give rise to these effects.

This is in strong contrast to visually-driven saccades, the dynamics of which seem to be vectorially encoded, resulting in approximately straight saccades.

In order to further study these apparent differences, we are currently recording the activity of saccade related burst neurons in the deep layers of the monkey Superior Colliculus during saccades towards auditory and visual targets.

Supported by MCoE II (6615) of the ESfR Initiative.

573.10 EFFECTS OF AUDITORY STIMULUS ON HUMAN GAZE SHIFTS TO VISUAL TARGETS. B.D. Cornell and D.P. Munoz* MRC Group in Sensory-Motor Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6

We investigated the effects of presentation of auditory stimuli on gaze shifts (eye-head movements) of visual targets in human subjects. Subjects were instructed to look as fast as possible from a central LED to an eccentric LED, either 40° to the right or left as presented on the computer monitor. Subjects were instructed to either select the same location as the eccentric LED (enhancer), or on the opposite side (distractor), or not at all (rest). The average saccade latencies are also shown.

The experiments were performed in a gap condition in which the central LED was turned off 200ms before onset of the eccentric LED. In this gap condition, subjects fixated the central LED until the onset of the eccentric LED, at which point a target was presented in either gap or enhancement condition. These experiments were also performed in a condition in which the central LED was turned on 200ms after onset of the eccentric LED. In this condition, subjects were able to saccade to the target at a rate of 0.9 Hz, which is comparable to their rate of fixation. The average saccade latencies are shown.

The data suggest that the oculomotor system is modulated by auditory stimuli in a way that is consistent with the hypothesis that the auditory system is involved in the control of gaze shifts to visual targets. Reaction times to acquire the eccentric LED were reduced significantly in all subjects during enhancer trials and increased significantly in all subjects during distractor trials. These experiments were also performed in a condition in which the central LED was turned off 200ms before onset of the eccentric LED. In this gap condition, subjects fixated the central LED until the onset of the eccentric LED, at which point a target was presented in either gap or enhancement condition. These experiments were also performed in a condition in which the central LED was turned on 200ms after onset of the eccentric LED. In this condition, subjects were able to saccade to the target at a rate of 0.9 Hz, which is comparable to their rate of fixation. The average saccade latencies are shown.

The results suggest that the auditory system is involved in the control of gaze shifts to visual targets. Reaction times to acquire the eccentric LED were reduced significantly in all subjects during enhancer trials and increased significantly in all subjects during distractor trials. These experiments were also performed in a condition in which the central LED was turned off 200ms before onset of the eccentric LED. In this gap condition, subjects fixated the central LED until the onset of the eccentric LED, at which point a target was presented in either gap or enhancement condition. These experiments were also performed in a condition in which the central LED was turned on 200ms after onset of the eccentric LED. In this condition, subjects were able to saccade to the target at a rate of 0.9 Hz, which is comparable to their rate of fixation. The average saccade latencies are shown.
574.1
A SYSTEM FOR NAKED-EYE NEAR-FIELD DEPTH PERCEPTION AND LOCAL VIRTUAL SPACE SCANNING OF TYPE II FORMATTED STEREOPAIR IMAGES: SUPPORTING STUDY FOR CLINICAL OPTOMOTOR STUDY - IN CHARGE: B. Hayashi, Dept. of Brain Function, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan and J.M. Krut, University of California, Los Angeles, CA, USA

574.2
VERGENCE FOLLOWING VERTICAL SACCADES IS UNDER ADAPTIVE CONTROL. Z. Kapoula, T. Eggert and F. Florentin, Lab. Physiol. de la Perception et de la Vision, CNRS-Colloque France, Paris, France. IDEES, Univ. Toulouse, France

574.3
SHORT-LATENCY DISPARITY VERGENCE RESPONSES IN HUMANS. C. Butzien, P. A. Miller, and B. J. Kruglein, Lab. Sensorimotor Research, National Eye Institute, Bethesda, MD 20892 and Dipartimento di Elettronica, Eletronica ed Informatica, University of Trieste, 34100 Trieste, Italy.

574.4
BINOOCULAR COORDINATION, STEREOSCOPIC VISION AND LISTING'S LAW. D. Tvedt, Departments of Physiology and Ophthalmology, University of Western Ontario, London, N6A 5C1, Canada.

574.5

574.6
A LONG-TERM STUDY OF EYE BLINK RESPONSES AFTER HYPOGLOSSAL-FACIAL ANATOMOSIS. A. Grütz*, A. Gunkel, W. F. Neles, E. Stuepmpt and J.M. Delgado-Garcia, Lab. of Neurosurgery, Faculty of Biology, Univ. of Seville, E-41012, Seville, SPAIN and I.E.T. Department and Institute of Anatomy, University of Cologne, D-50931, Cologne, GERMANY.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
574.7

ACTIVITY OF PONTINE OPM NEURON S DURING EYE BLINKS.

L. E. Mays* and D. W. Morris. Dept. of Physiological Optics and Vision Science Research Center, University of Alabama at Birmingham, Birmingham, AL 35294.

Pontine OPM Neurons (OPNs) are known to play a crucial role in the generation of saccades and quick phase eye movements. These neurons are located in the nucleus raphe interpositus of the monkey, near the midline and just ventral and rostral to the abducens nucleus. OPNs are tonically active in the awake monkey and cat and cease firing just before and during saccades and quick phases of nystagmus. OPNs have been shown to inhibit saccadic excitation and inhibitory burst cells, and electrochemical stimulation of the OPN region suppresses saccades and quick phases but not slow eye movements. Since blinks are often associated with saccades, it can be difficult to determine whether a cessation of OPN activity is associated with a blink or the eye movements which accompany a blink. In experiments using anesthetized monkeys trained to maintain fixation on a visual target, we have observed that OPN activity may be related to blink frequency and not simply to the eye movements which accompany the blinks. Furthermore, the duration of the pause is positively correlated with the duration of the blink. Analysis of the data obtained using lid coils shows that OPN activity ceases 10 ms before the blink onset and that the pause lasts considerably longer than the downward phase of the movement.

Supported by EY04693 and EY03089.

574.8

THE ULTRASTRUCTURE OF EYELID MOTONEURONS IN THE MACAQUE.

P.J. Mays* Departments of Anatomy, Neurology and Ophthalmology, Univ. of Mississippi Med. Ctr., Jackson, MS 39216.

Eyelid movements are primarily controlled by two muscles: the orbicularis oculi, which produces blink down-phases, and the levator palpebrae, which produces blink up-phases and moves the lid away from the eye during opening and closing. Neural control of these movements is mediated by motoneurons which control these two muscles. These motoneurons which control these two muscles have been identified with intraocularly transported WGA-HRP/HRP. The ultrastructure of these identified lid motoneurons and the axon terminals synaptically contacting them provide a basis for determining the pattern of inputs that produce blink and gaze-related limb movements. Levator motoneurons have a relatively low density of synaptic contacts upon the plasma membranes of their somata and proximal dendrites. In addition to their dendritic arbors, these motoneurons possess a high density of synaptic contact sites with the axons of other motoneurons. The synapses of these motoneurons are of the ocular variety: Orbicularis motoneurons have a high density of synaptic contacts on their somatic and dendritic plasma membranes, with terminals often forming continuous lines along some membrane segments. A characteristic feature of these motoneurons is the occasional presence of a subsurface system immediately beneath a synaptic contact. This subsurface system appears to be a dense-cored vesicle.

Supported by NIH grant EY07862.

574.9

EFFECTS OF SUPERIOR COLICULAR STIMULATION ON THE BLINK REFLEX IN MONKEYS.

S,M. La*, B Brezen, M. Basco, C. Evinger and JW. Gnad, Departments of Neurology & Behavior and Psychology, SUNY Stony Brook, NY 11794.

In alert Rhesus monkeys, the effects of SC stimulation on reflex blink responses were studied by delivering 100-300 ms trains of electrical pulses beginning 30-50 ms before a 30-50 ms air puff to one eye. Eye and lid position were monitored using the search coil technique. Location within the SC motor map was determined with 10-25 µA biphasic pulses at 250-300 Hz. The threshold current for blink suppression was always higher than for saccades. The most effective sites for blink suppression were in the rostral layers near the rostral pole, where the ratio of thresholds was roughly 1.5. This ratio increased to 3 or greater at superficial and caudal sites.

The gradient of stimulation within the SC correlates well with the gradient of excitatory projections of the SC to the brainstem oculomotor neurons (OPN). Moreover, stimulation of OPN suppresses blinks. Nevertheless, the difference in the threshold for producing blink suppression and saccades suggests that the underlying neuronal circuitry may differ for the two processes.

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574.10

BRANSTEN LINKAGE BETWEEN THE SUPERIOR COLICULARIS AND BLINKING.

M.A. Bassoe* and C. Evinger. Deps. of Psychology, Neurobiology & Behavior and Ophthalmology; SUNY Stony Brook, NY 11794.

Both clinical and experimental data show that swallowing can dramatically modulate the brainstem blink reflex circuit. Previously, we have demonstrated that the superrior colliculus (SC) is an important mediator of these effects, such that submaximal inhibition of the SC increases the blink. The goal of the current study was to identify the brainstem neurons that mediate the SC modulation of blinking. The hypothalamic oculomotor neurons (OPN) are one candidate because electrical stimulation of the OPN region (La et al., 1994) and stimulation of the rostral SC, a region which provides a large input to the OPN, is the most effective for suppressing blinks with electrical stimulation. However, since blink suppression with SC stimulation is higher than that for evoking saccades or producing fixation (La et al., 1994), it is also possible that a non-ocular group of brainstem neurons mediate SC blink suppression.

By presenting a single, electrical pulse to the SC in urethane anesthetized rats, we determined the latency of suppression of orbital ocular EMG (OOEMG) activity to be 7 ms. Single unit recordings in the brainstem revealed a group of tonically active neurons caudal to the OPN that paused with reflex blinks. Stimulation at the site of these neurons produced a decrease in OOEMG activity within 5 ms. Stimulation at other brainstem sites, however, produced blink suppression at much longer latencies.

Consistent with SC stimulation in monkeys and behavioral studies in humans, the SC appears to modulate reflex blinking through brainstem neurons other than those involved in saccadic eye movements.

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574.11

GRAVITY-DEPENDENCE OF TORSIONAL OPTOKINETIC GAIN IN RHESES MONKEYS.

D. Straumann*, M. Dieterich, V. Hens, BIM Hess, Neurology Department, University Hospital, CH-8091 Zurich, Switzerland.

In three rhesus monkeys we measured torsional optokinetic nystagmus using the three-dimensional search coil technique. The animals were exposed to 30 degrees of stimulation by an optokinetic sphere, which rotated about the naso-occipital axis at different velocities. Gain (eye-stimulus-velocity coefficient) during the last 5 seconds of stimulation was determined for upright and supine body position. Compared to stimulations in the upright position, we found that torsional gain of optokinetic nystagmus was strongly attenuated in the upright position with angular eye velocity saturating at 5-10 deg/s (see also Schaff et al. 1986 Soc Neurosci Abstr 12: 744). Gain values in supine position varied considerably among the three monkeys, and several investigators have briefly noted that OPNs are less effective for saccades than for torsional vestibulo-ocular reflex. The data can be interpreted that the velocity-storage mechanism active in torsional optokinetic nystagmus is supine position. In upright position, however, the rotation axis of torsional nystagmus does not coincide with the gravity vector. Hence, velocity-storage is inflated and torsional optokinetic gain decreases.

Supported by ESFRP (Munoz-II 6615 and NSF 31-31963.91)

574.12

LEAKY NEURAL INTEGRATION OF TORSIONAL VESTIBULAR SIGNALS IN HUMANS.


We investigated the dynamic properties of the human torsional vestibulo-ocular reflex (VOR) during roll head rotations, and compared them with those of the horizontal VOR movements. Differences were related to the contributions of the extracocular plant and the neural integrator. Three subjects underwent position-step stimuli in the roll plane, and the resulting torsional eye movements were recorded using the magnetic search-coil technique. Using optimal parameter estimation techniques, the data were fit to a step-response model of the torsional VOR, which incorporated plant parameters obtained through previous forced-duction experiments. Following the position step, the eye drifted back to is resting position in the torsional plane, demonstrating a time constant of neural integration typically ~2.5 sec. Despite previous evidence of an asymmetry in the ocular-motor plant between torsion and extension, torsional eye movements appear to be well yoked during roll stimulation.

When subjects underwent similar VOR stimulation in the yaw plane, the eye held their new horizontal position for a much longer period of time, always yielding a time constant of neural integration of much greater than 20 sec.

We hypothesize that the incomplete integration of vestibular signals for torsional eye movements reflects different neural properties. In addition, the yoking of binocular torsional eye movements despite possible asymmetries on the plant may demonstrate a larger separation of control of individual muscles than previously thought to hold true.

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THURSDAY AM
OCULOMOTOR: BLINKING, VENGEANCE, AND TORSION

574.13
THREE-DIMENSIONAL CONSTRAINTS ON COORDINATED EYE-HEAD GAZE SHIFTS IN THE MONKEY. D. Gitton* and J.D. Crawford, Montreal Neurological Institute and Department of Neurology and Neurosurgery, McGill University, Montreal, Canada, H3A 2B4.

We have studied the normal orienting behaviour of one head-free Macaca Fascicularis. Dual search coils were implanted in one eye, and temporally screwed onto the skull cap. Eye and head position questionnaires were recorded while the animal looked at visual targets presented randomly throughout the gaze range. With the head fixed, the eye was constrained to a Listing's plane with a torsional standard deviation of ±5°. Listing's plane was no longer maintained with the head free, because VOR slow phases produced torsional changes in eye position. However, opposite torsional components in the rapid eye movement components of the gaze shift prevented accumulated torsion. Both the head and eye-in-space loosely followed a strategy resembling a set of Fick gimbals (Glenn & Vills, J. Neurophysiol, 1992): horizontal rotations occurred about a relatively fixed vertical axis, but vertical rotations occurred about a horizontal axis that tended to rotate with the head. These axis tilts were not as stereotyped as those observed with the head fixed, and thus the ranges of head and eye position-in-space were not so tightly constrained (torsional standard deviations were 4° and 4.5° respectively). However, the ranges showed the curvature characteristic of the Fick gimbals strategy: Up-left & down-right gaze directions were accompanied by clockwise torsional tilt of the eyes & head, and down-left & up-right gazes had counterclockwise tilts (in earth-fixed coordinates). These results agree with human studies (Glenn & Vills, 1992), suggesting that the monkey provides an appropriate experimental model for invasive studies of this behaviour.

575.1
A NEURAL MODEL OF VOLUNTARY MOVEMENT AND PROPRIOCEPTION. D. Bullock*, P. Cleek, S. Grossberg, Boston University, Dept. of Cognitive and Neural Systems, Boston, MA 02215.

A neural model of voluntary movement and motor proprioception is developed within constraints from neuropsychology and motor psychophysics. The model generates trajectories to a desired endpoint in spatial coordinates, while using motor command corollary discharges and muscle spindle error feedback to construct an accurate perception of position. Response of model elements resemble observed cortical and subcortical physiological data while functional characteristics suggest explanations of proprioceptive illusions and load effects. Simulations show the system's ability to execute voluntary movements while reducing oscillations caused by limb dynamics and compensating for external forces such as those caused by loads or obstacles. Further simulations demonstrate how the network can self-organize its sensorimotor transformations through spontaneous co-contractions of synergistic muscles.

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575.2

Recent evidence suggests that motor programs are specified gradually (Hening W., Favilla M., Ghaz T.: Exp. Brain Res. 71, 116-128 (1988)), can be continuously updated, and are part of a closed feedback loop. The implied theoretical challenge is to address the dynamic properties of motor programs. We present a dynamic field theory of motor programs that is based on a neural representation of movement parameters such as movement direction and amplitude. The topology of such parameters is expressed by structuring the neural representations as fields. Motor programs are stable localized solutions in such neural fields. They evolve under two types of external influences: (1) localized input representing the perceptual specification of movement parameters and (2) input representing the pre-structuring of motor plans and thus reflecting the nature of the task space defined by the experimental paradigm. The distance of this pre-shape from the specified motor program in a dynamic sense determines the time needed to generate the correct motor program and therefore affects reaction time in the theory. We compare our model to experimental results of Ghez and colleagues on the gradual build-up of motor programs. We account for the Hick-Hyman law, the dependence of reaction time on the metrics of tasks, on pre-cued information, and discuss effects such as range or bias effects and deprogramming. The longer term goal of integrating such a dynamic description with a dynamic account of coordination (Schöner G., Kelso J.A.S.: Science 239, 1513-1520 (1988)) is sketched.

575.3

Why do humans shift from walking to running when speed increases? We have proposed a dynamic theory of human locomotion in which preferred gaits are attractors that exhibit stable phase relationships, and gait transitions are bifurcations characterized by a loss of stability (Diedrich & Warren, in press). According to this theory, manipulation of the location of the attractors should have an effect on the speed of the transition. As suggested by energy expenditure data, one way to manipulate this is by inclining the treadmill. Six participants walked and ran on a treadmill at grades of 0% and 10% while speed was varied. As expected, the speed at which transitions of the ankle-hip phase relationship were at a minimum shifted downward from 1.63 m/s on a 0% grade to 1.54 m/s on a 10% grade, consistent with movement of the attractor to a lower speed. Similarly, the mean transition speed on the descending and descending trials, shifted downward from 2.19 m/s at 0% to 2.00 m/s at 10%. In sum, changing treadmill inclination led to movement of the attractors and corresponding changes at the walk-run transition. There generally corresponds with those expected on the basis of previous energy expenditure data.

575.4
A NEURAL NETWORK MODEL OF BASAL GANGLIA THALAMOCORTICAL RELATIONS IN NORMAL AND PARKINSONIAN MOVEMENT. J.J. Contreras-Vidal*, G.E. Stelmach, Arizona State University, Laboratory of Motor Control, Tempe, AZ 85287.

Anatomical, neurophysiological, and neurochemical evidence support the notion of segregated parallel basal ganglia-thalamocortical motor channel. We developed a neural network model for the function of this system in simple and complex (sequential and simultaneous) movements in normal and Parkinsonian (PD) behavior. Non-linear differential equations are used to model the membrane potential for each cell type (short-term activation), its physiological action (inhibitory or excitatory), as well as neurotransmitter/neuromodulators (medium-term dynamics: e.g. GABA, enkephalin, substanina P). The model reproduces motor impairment in PD due to dopamine depletion such as akinesis, bradykinesia, and puts between movement components (the figure shows a normal and PD simulation of a two-stroke sequential movement). Specific observations of the effect of subthalamotomy and postero-ventral PD improvement are made. The model also suggests how the motor load is distributed across several brain structures during multi-joint movements. Support by Flins Grant, Contreras-Vidal on leave from Monterrey Institute of Technology (Mexico).
575.5 ACUTE EFFECTS OF LEVODOPA ON WRIST MOVEMENT IN PARKINSON'S DISEASE: KINETIC, VOLUMETRIC EMG MODULATION, AND REFLEX AMPLITUDE MODULATION MTJ Johnson*, A. Mendez, AN Kneiss, P. Silverman, F. Zwischel, A. T. Elger Departments of Neurology, Neuropsychology, and Physiology, University of Minnesota, and Minneapolis Clinic of Neurology, Minneapolis, Minnesota, USA.

Acute changes in motor performance due to levodopa were evaluated by a series of four motor tests unified by their focus on wrist flexion-extension movements. Subjects with idiopathic Parkinson's disease were evaluated with a test battery containing an isometric voluntary contraction in which velocity was to be maximized, visually guided tracking of a sinusoid and a square wave, and an array of stretch reflex modulation during volitional sinusoidal tracking. The maximal amplitude of active flexion and extension movements increased after levodopa (ON), without significant changes in the movement period or amplitude. In the two tracking tasks, the RMS error, peak velocity, or peak movement amplitude did not change after levodopa. Significant and consistent changes did occur in an array of reflex modulation during error-constrained tracking after levodopa. The amplitude of volitional EMG increased after levodopa, concurrently with a reduction in reflex EMG. These changes are consistent with the noted increase in movement velocity. These results show that the effects of levodopa on movement velocity were not translated into increased accuracy. Also, the changes in the long latency reflex gain argue for a central control of this reflex, mediated by structures sensitive to levodopa. Supported by NIH grants R44 NS28633 and T32 NS07361.


Target locations, as perceived by the visual system must be transformed into kinesthetic and motor coordinate frames for the upper limb to perform reaching movements. Recent studies in this laboratory have shown that the perceptually preferred anterior/posterior (AP) axis of kinesthetic coordinate system is trunk-fixed (Butler et al. 1993 - Soc. Neurosci. Abstr. 19, 550) and for the visual system is also likely trunk-fixed (Darling and Williams 1993 - AbS Proceedings of 17th An. Mtg., pp 110). The earth-fixed gravitational axis is preferred by the kinesthetic system at the perceptual level (Darling, manuscript in preparation). The purpose of this investigation was to determine if the visual system prefers the earth-fixed gravitational axis to body-fixed axes at the perceptual level. Ten normal healthy individuals positioned a hand-held lighted wand under four different experimental conditions that were each run under light and dark conditions. The earth-fixed gravitational axis was moved in anatomical positions and moved the wand parallel to the gravitational axis, (2) earth - same as (1) except that subjects head and trunk orientations were varied, (3) head - same as (2) except that subjects positioned the wand parallel to their head-fixed longitudinal axis and (4) same as (2) except that subjects positioned the wand parallel to the trunk-fixed longitudinal axis. Orientations of the head, trunk and wand were recorded optoelectronically. The results clearly showed that subjects were most accurate at positioning the wand parallel to the gravitational axis even when head and trunk orientations were varied. Control experiments showed that kinesesthesia was not used for precise positioning of the hand-held wand. Thus, the visual system prefers the earth-fixed gravitational axis to body-fixed longitudinal axes at the perceptual level and coordinate transformations at this level are simplified because of the parallel axes for the visual and kinesthetic coordinate systems.

575.9 FOCAI INTRASPINAL NMDA IONTOPHORESIS PRODUCES DISTINCT ACTIVATION PATTERNS IN THE FROG, P. Salonit and E. Bizzi. Dept of Brain and Cognitive Science, MIT, Cambridge, MA 02139.

In the spinal frog, microinjection of the lumbar grey produces only a few possible orientations for the convergent force fields recorded in the horizontal plane from the isolated hindlimb muscle. This force field points towards body, rostral flexorward extensor and medial extensor. To determine if this representation activates fibres of passage or of neurons, and as a preliminary step to anatomical studies aimed at identifying such fibres, NMDA (final concentration 250 nM) was iontophoresed at eight sites within microinjection tracks within the same pipette that had been effective. Forces and 11 EMGs were recorded. 38% of sites (32 sites in 7 frogs) did not respond to NMDA. Of the NMDA-responsive sites (average latency 16 sec., range 4-29 sec.), the forces evoked by chemical stimulation had the same direction of orientation as those visually, matching them at 68% of sites. For the EMGs, a close match was seen in only 38% of cases.

However the NMDA-evoked EMG's fell in a few distinct patterns which could be related to the force produced. One pattern (1/17 sites) consisted of a nearly simultaneous onset of a tonic or rhythmic oscillation of all muscle except for RA (rota anterior), VI (vastus internus) and GA (gastrocnemius) which were only weakly activated. With one exception towards body forces, a second pattern (6/17 sites), muscle recruitment occurred progressively over a succession of cycles, with the production at first of rostral flexion and then flexor body forces. RA, VI, and GA were among the most active muscles. Cose sites eliciting the first and second patterns were generally located caudally and rostrally to the 8th root respectively. A third pattern (2/17 sites) produced outward extensor forces. In conclusion, focal intraspinal NMDA iontophoresis may be helpful to localize groups of neurons responsible for the generation of distinct EMG patterns which produce the same set of force orientations previously identified with electrical microstimulation. (Supported by ONR N00014-94-K-0273)

575.6 THE EFFECTS OF GRAVITATIONAL CUES ON THE TRANSFORMATION BETWEEN KINESISTHETIC AND VISUAL INFORMATION TO REACHES TO REMEMBERED TARGET LOCATIONS: A. Butler*, W.G. Darlington, M.A. Frazzetto. Department of Exercise Science, University of Iowa, Iowa City, IA. 52242.

The abilities of human subjects to point to kinesthetically presented targets which are no longer present during a reaching movement was studied under 2 gravitational and 3 reaching conditions: (1) VS/G, VGS - visual self motion; (2) GS/G, GVS - no motion, no gravity; (3) VS/VG, VVS - visually reach with pointer, no gravity. The ability to specify target locations on the basis of kinesthetic information alone has been found to be accurate (Darling and Miller, Exp Brain Res. 1993, Vol 93, pp 534). Reaching with a pointer requires transformation of kinesthetically derived limb coordinates into a visual coordinate system. Availability of gravitational information about target locations was not manipulated in previous studies and is thought to be used in the kinesthetic representation of upper limb orientation. Thus, the main purpose of this experiment was to study the abilities of subject to reach to a remembered kinesthetic target location with or without gravitational information on target location. We presented 10 targets in two planes using a programmable arm. Positions of the arm, hand, fingertip and target object in three-dimensional space were recorded using optoelectronic techniques (WATSMART system, Norhem Digital). Manipulating gravitational information on target location had little influence on directional and distance errors during visually guided reaching with the hand. When using the pointer, there was a substantial increase in distance errors, but not in direction errors. Availability of gravitational information had little influence on a performance when reaching with the pointer. Consistent with the findings of previous work, subjects tended to overlook close targets and underestimated far targets during visually guided reaching to kinesthetic targets. These results suggest that removing gravitational information about kinesthetic target location does not interfere with reproducing a kinesthetically remembered hand location or with transformation of kinesthetic information into a visual coordinate system.


The attraction of motor control to oscillatory components is one of the fundamental features of biological systems. Recently, we reported a systematic phase relationship between tremor and the onset of fast voluntary motor responses in patients with Parkinson's disease (PD) (Staudte, C. et al. Soc. Neurosci. Abstr. 1982:1862). Experiments with control subjects mimicking tremor showed similar results. The aim of this study was to establish a functional model for the attractive effect of the tremor oscillator in the framework of the equilibrium point (EP) hypothesis. According to the EP hypothesis, there is only one central variable that controls the behavior of a muscle, the threshold λ of its tonic stretch reflex (Feldman, AG Biophysics. 1966:11:565-578). Single joint movement models are induced by a shift (virtual trajectory) in control variables λ, of at least one pair of antagonist muscles from an initial value (that depends on the actual phase of the tremor cycle) to a final state. Within the virtual trajectory model, the movement trajectories and the electromyographic patterns of the antagonist muscles can be predicted. Movement patterns of PD tremor patients and control subjects mimicking tremor were recorded under both isometric and kinetic reaction time paradigms and compared to computer simulations. The EP hypothesis proved to reliably predict the observed movement patterns under various conditions. We conclude that attraction of voluntary motor response to intrinsic oscillators is a system inherent phenomenon of motor control and does not depend on the origin of the oscillatory component.

575.10 MOTOR TEMPLATES IN TYPING: F. Viviani and G. Laisard. 1Faculty of Psychology and Educational Sciences, Univ. of Geneva, Geneva, Switzerland and 2Dept. of Cognitive Science, Istituto S. Raffaele, Milan, Italy.

We investigated the performance of professional typists transcribing texts presented visually. The timing of the keystrokes was analyzed as a function of the following factors: 1) the combination of fingers used for typing digrams; 2) the relative frequency of the digrams in the language; 3) the word-context in which digrams are embedded; 4) the total duration of the typing sequence for the word. The mechanical and linguistic factors were found to influence the interstroke intervals. However, over and above these factors, we demonstrated that words (i.e. well-formed lexical items) were typed with sequences of keystrokes whose temporal structure depended idiosyncratically on the words and the typing context. Within the range of variability of the rhythm that is normally observed in transcription, this structure - defined as the set of all ratios between interstroke intervals - remained unchanged (homothetic invariance). The results suggest that these highly complex motor sequences are controlled by global motor plans that include an abstract representation of the timing of the keystrokes. A simple counting mechanism was postulated to explain the findings. We contrasted four variations of the mechanism on the basis of the pattern of temporal variability.

Previous work from this laboratory has described a set of movement primitives encoded in the frog spinal cord. In order to examine the generality of these findings, we are currently exploring movements encoded in the spinal cord of the rat. As a first step in this project, we describe here the pattern of position dependent forces, i.e. the force fields, produced by rat hind limb muscles and the result of combined stimulation of two muscles. In the frog, simultaneous stimulation of two muscles produces a force field which is the vector sum of the force fields of the two muscles stimulated separately. This property of summation holds for the non-redundant case, with the force transducer attached to the ankle, and not for the redundant case, with the force transducer attached to the foot. We found a similar pattern of results in the rat: the force field predicted by summation of the two separate muscle fields was highly correlated with the field produced by the costimulation of the muscles for both the redundant and non-redundant case. This result implies that, at least for the degrees of redundancy in these experiments, the combination of muscles can be approximated as a linear vector sum, potentially simplifying the control of the hindlimb. Supported by NIH NS09043 and AR05710 and ONRNO0014993/1946.


Tonic electrical stimulation to the midbrain periaqueductal gray (PAG) induced naturally sounded vocalization. The stimulation to the pontine call site (PCS) within the ventrolateral pons also induced vocalization. To know whether the PCS is the descending pathway for vocalization from the PAG to the lower brainstem, an anterograde tracer, Phaseolus vulgaris leucoagglutinin, was applied at sites located in the lateral PAG where the electrical stimulation induced vocalization. At the level of the caudal superior colliculus, labelled fibres sparsely distributed and passed laterally through the dorsal part of the midbrain tegmentum where the stimulus threshold for vocalization was higher than the PAG. At the level of the inferior colliculus, they could be traced ipsilaterally to the ventral area along the medial lemniscus and consisted of a narrower bundle around the PCS where the stimulus threshold was the lowest.

At the level of the upperpons, they occupied a broad but thin region in the dorsal portion of the trapezoid body ventral to the medial lemniscus. Further caudally, they appeared to be concentrated in a discrete area ventral to the inferior olive and dorsal to the pyramidal tract, and could be continuously traced to the upper cervical spinal cord.

It was known that the PAG stimulation induced vocalization associated with rage, while the PCS stimulation induced vocalization without rage. The present results suggested that the PCS is the descending pathway specific for vocalization from the PAG to the lower brainstem.

MOTION SICKNESS HELPS PRESERVE SENSORMOTOR COORDINATION IN THE PRESENT AND FUTURE. D.W. Jensen*. Department of Biology, Tomball College, Tomball, TX 77375.

A new hypothesis is proposed for the function of the mechanisms of motion sickness. The hypothesis accounts for data from plasticity of postural control, development of sensory-motor coordination, motion sickness, vomiting, pharmacology, and neurotendy hypothesis. The mechanisms giving rise to the early symptoms of motion sickness impel an individual to remove the sensory conflicts of an offending motion. The early symptoms are a variety of ill feelings and actions that do not interfere greatly with motor control. The sufferer can still remove sensory conflicts, so the brain is not stimulated to undergo long-term adaptive changes that alter sensorimotor coordination. Thus, the early symptoms and their mechanisms serve to resist redefining normal motion while concordantly preserving the existing sensorimotor coordination. Later developing symptoms of vomiting and severe nausea, however, impose motor control problems; in such a state one cannot control much of anything. This extremely ill feeling is the same as what is produced by certain neurotoxins. The state of being poisoned by provocative motion is strongly associated with the motion. When the same kind of sensorimotor-alteration-motion occurs again that association is recalled. Acting on this recall and avoiding that motion preserves the existing sensorimotor coordination in the future.

THE ROLE OF DOPAMINE TERMINALS IN THE NUCLEUS ACCUMBENS IN LOCOMOTION INDUCED BY ACTIVATING THE HIPPOCAMPAL → ACCUMBENS GLUTAMATE PROJECTION.


Earlier electrophysiological evidence has shown that the stimulation of the hippocampal input to the nucleus accumbens evokes short-latency, early-duration, glutamate-mediated excitatory responses in the nucleus accumbens neurons (Yang & Mogenson, 1984). It has also been shown in our recent behavioral studies that injecting glutamate receptor agonists, NMDA or AMPA, into the nucleus accumbens activates the glutamate receptors located on mesolimbic dopaminergic terminals (Wu et al., 1993). This evidence suggests that glutamate in the nucleus accumbens may act on glutamate receptors in mesolimbic dopaminergic terminals and accumbens output neurons. The objective of this study was to investigate the role of the mesolimbic dopamine terminals in the nucleus accumbens in the initiation of locomotion by activating the hippocampal excitatory input to the nucleus accumbens.

Locomotion (measured in an Opto-Varimex-3 activity cage) was significantly increased by unilateral injections of NMDA into the ventral subiculum of the hippocampus. This NMDA-induced locomotion from the hippocampus was abolished after the destruction of the mesolimbic dopaminergic terminals in the accumbens, by injecting 6-OHDA into the ventral tegmental area (VTA). The results suggest that the mesolimbic dopamine terminals are essential in transmitting hippocampal signals to the output neurons within the nucleus accumbens.

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ACTIVITY IN VENTROLATERAL THALAMIC AREAS DURING CONDITIONED VOCALIZATIONS IN CAT. G.R. Farley*, Research Division, Boys Town National Research Hospital, Omaha, NE 68131.

Although ventrolateral thalamus has been implicated in motor control tasks, its role during vocal behavior might be questioned. Jurgens and colleagues, based on electrical stimulation and lesion data, indicated that it was part of a core system of brain structures capable of producing vocalizations in the absence of concomitant reinforcing potentiation. In the present study, we recorded single- and multi-unit activity from sites in the ventrolateral thalamic area in awake, behaving cats. A substantial proportion of recording sites showed strong temporal correlations in neural activity with various aspects of the instrumental conditioned vocalization paradigm, including both vocal production and feeding behavior. Activity related to feeding was generally strongest, and most prevalent, typically involving bursts of firing during ingestion of the food reward. However, there were also numerous examples of increased and decreased firing during vocalizations, as well as changes in firing rate immediately following vocalization. There were no obvious changes in firing related to background respiratory activity. However, a number of loci showed bursts of activity apparently related to laryngeal EMG activity not associated with vocalizations. Finally, we also noted apparent long-term fluctuations in background firing rate associated with periods of intense behavioral activity versus periods of behavioral quiescence. These data are consistent with a possible role of this structure in motor control during vocal production. (Supported by NIH grant 5-R01-DC01535-01.)

ESTIMATION OF IDENTIFIED INTERNEURONS FORMING A FUNCTIONAL GROUP CONTROLLING ABDOMINAL POSITIONING IN THE CRAYFISH, P. Boesiger, and J.L. Larimer*. Dept. of Zoology, Univ. of Texas at Austin, Austin, TX 78712.

Several studies have indicated that the command elements controlling abdominal positioning (flexion and extension) in the crayfish can predict the abdominal positioning was observed in isolated nerve cords by recording from motor roots while stimulating single command elements intracellularly. Extracellular recordings of interneurons were made from rostral and caudal connectives or connectives* (1 and 2). When one of these command elements is stimulated it activates a number of previously defined elements and elements increases or inhibits the spontaneous activity of other interneurons. We then compared spontaneous with evoked activity. The largest number of recorded interneurons was 64, the smallest was 4, and the average was 23 (r=15). Some of these command elements are identified cells based on morphology and physiology. Data was gathered from one identified cell on three separate occasions. A comparison of these data revealed that although the number of recorded cells and their firing frequencies were not identical they were surprisingly similar. These data support the functional group hypothesis, and suggest that the number about 23 interneurons controls each abdominal positioning behavior.

Furthermore, these data and previous studies indicate that the interneurons within a group may or may not be tightly coupled to each other, and their recruitment may be based on such factors as stimulus intensity and synaptic efficacy. Counts from other hemiconnectedes were doubled since motor output and nerve cord activity are usually synergically. (Supported by NIH grant NS 54243, to J.L.L.)
575.17

SEPARATE PERIPHERAL NERVES INNERVATE THE HEADS OF THE HUMAN LATERAL PTERYGOID MUSCLE. M. A. Aziz and R. J. Conne *

Anatomical, developmental and biomechanical studies of the human lateral pterygoid (Lp) muscle indicate that it is divisible, at least partially, into two heads. Electromyographic (EMG) studies supporting the independent activity of the superior (SLP) and inferior (ILP) heads during the masticatory cycle have been questioned. The precise peripheral nerve pathways to the two heads and the full structural complexity of the masticatory apparatus remains uninvestigated. Therefore, we have greatly dissected the Lp muscle and nerves, and measured the fiber orientations of 7 dissection room specimens. Photographs, drawings, and whole sections through the masticatory space were analyzed. Our results show that: i) the two heads are independently innervated, such that the SLP receives a single branch from the root of the masticatory nerve, while the ILP receives two or more branches from the root of the long buccal a., as well as loops bridging the anterior and posterior mandibular (V3) divisions; ii) the origins of the heads are easily recognizable, but in two cases each head shows further horizontal segmentation; iii) the mean insertion orientations of SLP and ILP fibers are 27° (±9) and 38° (±9) from the Frankfort plane, respectively.

These findings support: i) a claim that the two Lp heads are enclosed in separate fascial compartments; ii) EMG studies indicating their independent functions; iii) the likely segregation of their central control mechanisms. These data are prerequisite to a planned histochomical demonstration of the latter in primates.

575.19


Negative phototaxis in the river lamprey (Lampetra fluviatilis) was characterized behaviourally, and the strategies for performing yaw turns away from light were investigated with video recordings, combined with EMG-recordings from the myotomal musculature, in freely behaving animals. In swimming lampreys, illumination from one side evoked a yaw turn away from the light. An increase in light intensity resulted in a significant increase in yawing angle. Strong lateral illumination frequently evoked an escape response, characterized by a decrease in swimming speed, an approximately 180° yaw turn either away from or towards the light, and subsequent locomotion in a direction opposite to the initial one. During yaw turns the locomotor rhythm was maintained and asymmetrical, so that the lateral displacement of the undulatory wave was larger in the direction of the turn, and smaller on the other side. In the EMG-recordings, the intensity and duration of individual muscle units were highly variable also during straight swimming, which presumably reflects variations in the activity of individual motor units between cycles. Stronger turns (> 60°) were correlated with an asymmetrical increase in burst intensity and duration on the ipsilateral side, and a slowing down of the rhythm. During weak turns no consistent changes in the EMG-pattern were seen. Eye illumination of quiescent lampreys, attached with their suckers to the bottom, evoked a strong yaw turn away from light, and locomotion. The initial turn was reflected in the EMG-recording, as a unilateral burst of high intensity and long duration. Burst intensity was positively correlated with the turning angle.

575.20

AN ASYMMETRICAL VISUAL INPUT CAN ABOLISH POSTURAL DISTURBANCES EVOKED BY UNILATERAL LABYRINTHOTOMY IN THE LAMPEREY. T.O.Delphinia * Nobel Institute for Neurophysiology, Department of Neurosciences, Karolinska Institute, 171 77 Stockholm, Sweden.

In all classes of vertebrates, removal of the vestibular organ (unilateral labyrinthectomy, UL) results in severe motor disorders. In the lamprey (a lower vertebrate), one of the most pronounced symptoms induced by UL is continuous rotation (rolling) around the longitudinal axis during swimming. The aim of the present study was to abolish this symptom by means of an artificially organized, asymmetrical visual input. Five lampreys, with two eyes surgically removed from their finding that an asymmetrical visual input can strongly influence both the lamprey-driven roll control system (Delphinia et al., Exp. Brain Res. 95, 421-428, 1993). Swinging of the lampreys subjected to UL (control), and the effects produced by different types of asymmetrical input combined with UL were investigated. The animals of the first control group (UL only, n=11) rotated continuously during swimming. This symptom lasted up to 12 weeks after surgery. In the animals of the second group (UL plus bilateral inclusion, n=14) rotation during swimming was observed even longer, for 6-10 weeks. However, if a removal of the labyrinth was accompanied by a removal of the eye on the same side, the animals (n=17) swam normally, without rotation, immediately after surgery. In animals subjected to UL and bilateral inclusion, which were rotated continuously, the rotation terminated immediately with onset of electrical stimulation (10Hz) of the optic nerve ipsilateral to the intact labyrinth (n=5). In the lamprey deprived of the labyrinth and the eye on the same side, input from the remaining eye was able not only to functionally substitute the lost vestibular input, but also to induce adaptive changes in the roll control system: a removal of the remaining eye in 10-30 days after the first surgery did not destabilize the postural control, and the animal driven by only one labyrinth swam normally, without rotation.

575.21

MASTICATION FUNCTION IN PATIENTS WITH CORTICAL BRAIN INJURY AND HEMIPLAGIA. N. Kemppainen*, J. Waltimo, M. Kaste, H. Palosuo and 0. Saalonen. Dept. of Prosthetic Dentistry, Univ. of Helsinki, Dept. of Neurology and Dept. of Radiology, Helsinki Univ. Central Hosp., Finland.

The masticatory function of sixteen patients (11 men and 5 women) with severe hemiparesis caused by brain infarction (A. cerebri media) was studied by means of interview, clinical examination and bite force measurements. The location of the infarction was assessed with computer tomography and magnetic resonance imaging to be localized in the facial primary motor cortex. The Rankin Disability Score and the Scandinavian Stroke Group Index showed that the patients had an infarction causing marked invalidity, eg. facial paralysis and a marked loss of hand grip force on the contralesional side of the cortical lesion. However, the patients reported no change in their chewing ability, although four of them reported hoarding of food in the buccal sulcus area of the paralyzed side. The clinical examination revealed no major signs of cranial and motor disorders, and masticatory muscles contracted symmetrically when examined by palpation. Bite force measurements also indicated symmetrical contraction, since we could not detect any difference in the maximal bite force between the healthy and paralyzed side. These results are in agreement with recent laboratory findings in monkeys supporting the importance of bilateral representation and cortical projections in the production and coordination of orofacial functions.
567.1 MULTI-JOINT COORDINATION DURING THE WIPING REFLEX IN FROGS.
M.G. Sirota, R. Dubois and A.O. Feldman.
CéSNN, Université de Montréal H3C 3J7 and Dep. de kinésiologie, Université du Québec à Montréal, Québec, Canada H3C 3P8.

Intact and spinal frogs produce non-rhythmic target-directed hindlimb movements in response to stimulation of the skin (the wiping reflex or WR). Analysis of the WR allows us to study the problems of redundancy in terms of degrees of freedom. We studied the redundancy problem by analyzing interjoint coordinations with kinematic techniques for the WR in response to central body surface stimulation in grass frogs (n = 9). These frogs were spinalized between the 1st and 2nd vertebrae under tricaine anesthesia. WRs were elicited by a series of 3-5 light pricks with a needle. Hindlimb movements were recorded in two dimensions using a light-reflecting markers (8 points on the hindlimb joints and body). Movement duration was effectively increased by cooling the projection. Trajectories of the limb's endpoint and angle-angle diagrams were analyzed. Results show that there are phases of the WR in which a significant excursion in a single ("leading") joint is associated with relatively small excursions in the others. The leading joint can be different for different phases of the WR. However, there are phases in which joint angles change simultaneously. In the later case, the coordination between joints was not fixed and might be changed in repeated movements. It is concluded that target-directed reaching can be accomplished by relatively independent movements in the joints despite motor task constraints.

Funded by a Group Grant from MRC Canada and FRSQ Quebec.

567.2 ORGANIZATION OF CENTRAL CONTROL SIGNALS ASSOCIATED WITH MULTIPLE DEGREE OF FREEDOM ARM MOVEMENTS. L.E. Sergi* and D.J. Ostry.
McGill University, Montreal, PQ, Canada H3A 1B1

To gain insight into the organization of central control signals associated with motion in one or more degrees of freedom, we examined the behaviour of bi-articular muscles during movements in which they acted as agonists and antagonists, and acted as antagonist to another. As an example, the action of biceps brachii, which acts to flex and supinate the forearm, was studied during a series of flexing pronations, where it acted as an agonist to the flexion movement and an antagonist to the pronation movement. We addressed the following question: Will bi-articular muscles display purely agonist or antagonist activity depending on factors such as movement amplitude or required torque, or will the muscle display antagonist activity. Subjects performed flexions or extensions of a fixed amplitude while simultaneously performing supinations or pronations of increasingly larger amplitude. The activity of eight muscles about the elbow was recorded using surface electrodes while arm position was monitored using OPTOTRAK. Results show that bi-articular muscles display an activity pattern which has both agonist and antagonist components. For example, during a small flexion combined with a large supination, pronator teres, which acts to flex and pronate the forearm, displays phasic activity concurrent with brachiais, a pure agonist. It also displays a burst of activity concurrent with pronator teres, another antagonist. This suggests that central commands for motion in individual degrees of freedom may be specified independently and superimposed.

567.3 EXAMINATIONS OF POSSIBLE EXPLANATIONS FOR TRAJECTORY CURVATURE IN MULTI-JOINT ARM MOVEMENTS. Oto, R.*1, Uno, Y.1, Kojge, Y.2, & Kawato, M.2
Dept. of Psychol., Fac. of Letters, Kyoto Univ. & ATR Human Inf. Processing Res. Labs., Kyoto, Japan.

We discuss the coordinate frame in which visually guided human multi-joint arm movements are planned. In point-to-point planar reaching movements, hand paths tend to be gently curved. Models that rely on intrinsic-dynamic coordinates such as the minimum-torque-change model predict curved paths whereas models that rely on extrinsic-kinematic coordinates such as the minimum-jerk model predict straight paths. To decide between these two coordinate frames, we tested following three possible explanations for observed curvature in extrinsic-kinematic models (Wolpert, et al., 1993): (1) The planned trajectories are straight but imperfect execution of control causes the curvature. (2) Visual misperception causes the curvature. (3) Arm trajectories are controlled using straight virtual trajectories and biomechanics of the arm causes the curvature. In Exp. 1 we examined the movements from the side of the body to the front of the body. We found that if subjects were explicitly instructed to generate straight paths for such movements, these movements were straighter than those generated spontaneously. This result argues against (1). In Exp. 2, subjects generated spontaneously curved trajectories even in the fronto-parallel plane where visual misperception was not expected to be a factor. This result argues against (2). In Exp. 3, EMG signals of 6 related muscles of the same movements as Exp. 1 suggest that subjects can make straighter paths without raising stiffness. This poses a problem for the portion of (3), namely, the virtual trajectory hypothesis that accounts for the performance of straighter trajectories using straight virtual trajectories by raising stiffness. Thus our results support the hypothesis that the CNS plans trajectories using intrinsic-dynamic coordinates. Supported by JSPS Fellowships for Japanese Jun. Scientists.

567.4 LEARNING AND TRAJECTORY PLANNING IN KINEMATIC ALTERATION OF JOINT ANGLES. H. Imamizu*, Y. Uno and M. Kawato
ATR Human Information Processing Research Labs, Kyoto, Japan.

We investigated whether trajectories of the human arm are planned solely in an extrinsic (kinematic) space or in both an intrinsic (dynamical) space and an extrinsic space. We virtually misaligned (1/2) the elbow angle and magnified (5/4) the shoulder angle of normal human subjects while they were aiming at targets. A position marker was attached to the subject's hand and its current altered position was displayed as a cursor on a CRT screen. This linear transformation in joint angles corresponds to nonlinear transformation between the hand plane and the screen, and makes a straight trajectory on the hand plane a curved one on the screen. If trajectories are planned in an intrinsic space then they are invariant in the intrinsic space (the hand plane) and distorted in the extrinsic space (the screen) under the kinematic alteration. On the other hand, if they are planned in the extrinsic space, they are invariant in the extrinsic space and distorted in the intrinsic space. The task for the subjects was to move the cursor to a target within a short time period (900 ms). The aiming error decreased with practice. We compared the trajectories after 320 trials of training to those under no alteration on the screen. There was significant difference between them. This suggests that the trajectories were distorted in the extrinsic space. The result supports the hypothesis that trajectories are planned in the intrinsic space. We also investigated intermanual transfer of the learning effect to obtain evidence of internal representation of intrinsic coordinates (joint angles) in the central nervous system.

567.5 The Three-dimensional Curvature of Unrestrained Straight-ahead Movements. F. E. Pollick*, C. Ishimura
ATR Human Inf. Processing Res. Labs, Kyoto, Japan.

We examined the three-dimensional curvature of unrestrained point-to-point hand movements in the forward direction. Subjects moved the hand from a position above the start point to a forward position above targets of different size and distance. Paths showed curvature resulting from an initial lateral and primarily downward movement that was compensated for in the second half of the movement. The power component of motion was temporally coupled to the forward motion, with the maximum drop occurring at the time of peak forward velocity. The curvature was greatest for movements that aimed for targets at intermediate range and decreased as target distance increased. Analysis of the relationship between velocity and radius of curvature showed that velocity was related to radius of curvature by a power law with an exponent of 0.59, different from the value of 1/3 typically obtained in curvilinear drawing motions. Aspects of the downward motion suggest that it was purposeful in speeding up the movement and offer explanation to the deviation from the typically reported exponent.

567.6 LEARNING AND GENERALIZATION OF LIMB DYNAMICS.
R.L. Sainburg & O. Ghez.

We have recently shown that impaired control over interaction torques in proprioceptive deafferentation can be dramatically reduced when, prior to movements performed without vision, patients were allowed to view their limb in motion. We therefore proposed that visual updating of a model of limb dynamics used to program movement. We now ask whether intact subjects also use feedback to control interjoint dynamics and examine the learning and use of internal limb models.

Subjects were to trace linear hand-path templates presented on a computer screen with rapid, overlapping out-and-back movements. Their arms were supported in the frontal plane by a low inertia one-degree-of-freedom system. An outlier to which weights could be attached enabled the distribution of mass of the forearm-hand segment to be varied. Subjects were trained in a given direction of movement, with a given inertial configuration, by providing them with visual feedback of the screen cursor. Accuracy was then tested during movements in other directions and with different inertial configurations.

After training, subjects made errors in initial direction, movement linearity and interjoint coordination. Simulations showed these errors resulted from the production of torques appropriate to the inertial conditions during training. Such effects also occurred for movement directions that differed from the training direction. Accuracy improved as the magnitude was greatest in the trained direction. We conclude that the normal control of interjoint dynamics is dependent on feedback mechanisms that employ internal models of limb mechanics that can be rapidly recalibrated. However, recalibration through training in a single direction allows only incomplete generalization to untrained directions. (NS22715).

The present study examines the spatial control of distal joint angles when interaction forces produced by the motion of proximal joints are reduced. Cats reached to grasp a piece of beef from the end of a narrow food-well at different inclinations and heights. The coordinates of markers at the shoulder, elbow, MCP and 4th digit were recorded at 100Hz for kinematic and dynamic analysis. Joint torques were partitioned into components due to the effects of segments distal to the joint, intersegmental interactions, gravity and a residual term that includes active muscle contraction and passive visco-elastic resistance.

Paw paths were compared on two linear segments in which the paw was aimed first to a vis point in front of the food-well (lift), and then, within the well, to the frontal plane. During lift, kinematic trajectories were compatible without training, and hand movement and hand muscle activity so distal angles stayed independent of reach height. This indicates that there is a bias for muscle and joint torques to distal joints, to vary independently of movement height. Training to control the contraction required of distal muscles (NS31391).


We have previously shown that direction and extent of hand movement represent independently planned features of reaching movements. The purpose of this study was to compare the time courses of adaptation to experimentally imposed rotations and gain alterations in the virtual workspace. Healthy adult male movements were recorded on a horizontal digitizing tablet. However, vision of the hand and arm was blocked, and virtual targets and hand positions were displayed on a computer screen. We separately applied either a rotation or a change in gain to the screen display of hand path while leaving the virtual targets unchanged. The hand display was used to plan the path taken by the virtual hand was displayed following each movement as knowledge of results.

Adaptation to both types of distortion showed evidence of two distinct processes: a relatively rapid reduction in mean error over trials, and a more gradual reduction in variability of movement error. Adaptation to rotational distortions, and re-adaptation to conditions after rotations, was generally slower than for gain changes. Increased variability was also more pronounced and prolonged during adaptation to rotational distortions.

Differences between directional and extent adaptation support previous findings of independent specification of direction and extent. The learned visuo-motor transformation between target and hand direction is generally robust to short-term adaptation, perhaps because this involves altered specification of which muscles to contract. The target-extent relation is more rapidly recalibrated, perhaps because it involves shifting a scaling factor. (Supported by HD 01018, NS 22715).


We have previously reported that when subjects reach to visual targets without seeing their limb, movements show directional biases that vary with the initial position of the hand. The spatial organization of these biases suggest that planning movement direction the nervous system underestimates the distance of the hand from a bias-free position between midline and the target. Since this is the horizontal workspace of the hand, we hypothesized that these errors reflect their prior experience. We now asked: (1) Can directional bias in a specific location be eliminated by training with visual feedback? (2) Does such training alter the biases in other untrained areas of the workspace? Subjects moved their hand on a digitizing tablet from different central points to 12 radial targets displayed on a computer screen. Subjects were trained to perform accurate movements from initial positions normally associated with clockwise or counterclockwise biases by providing visual feedback of cursor and hand during movement. They were then tested without feedback for movements initiated from the same and other points. Training signified directional biases for movements initiated in the region of training. Movements initiated from other locations, including the previous error-free region, showed new biases that again represented underestimates of the distance of the initial hand position, but from the new trained location. The present findings are compatible with the hypothesis that training in a particular region of the workspace resets subjects' default estimate of their initial hand position. Kinematic planning is richly dependent on learned representations of the hand in the workspace. (NS 22719).


Learning a novel movement pattern requires developing a new dynamic model of limbs and objects ("getting into the ball park" Greene, Prog Theor Biol. 1972). It is proposed that initial learning is enhanced under consistent practice conditions which permit stabilization of the dynamic model and disrupts learning. These hypotheses were tested with similar tasks (SIM): using chopsticks to pick up and transport small, medium or large buttons to a container; or dissimilar tasks (DIS): chopsticks/medium button, card sorting, mirror tracing. Adult subjects (N=36), naive in chopstick use, were assigned to one of 4 practice conditions: Blocked/SIM (15 successive trials on each of 3 tasks randomly ordered), Blocked/DIS, Random/SIM, Random/DIS. Movement time was tested (medium button), immediate and delayed retention tests and 2 transfer tests (new button sizes). Findings confirmed predictions: Blocked/SIM yielded better learning than Random/SIM practice. DIS conditions did not differ in retention but tended to be better than Random/SIM. These results contradict reports (Magni & Hull, Q.J.E.P, 1999) favoring random practice over blocked ("contextual interference effect").

576.11 THE RELATIVE CONTRIBUTIONS OF GEOMETRIC VERSUS BEHAVIORAL CONSTRAINTS IN RESOLVING JOINT-LEVEL, MOTOR-EQUIVALENCE. Rebecca A. Stanley* and Charles F. Wight. Psychology Dept., Columbia, University, New York, NY 10027.

Motor equivalence exists when a task may be performed using various movement patterns. For example, in a pinch task, all arm configurations that suffice to position the hand at a desired location constitute a "motor-equivalence set." Motor equivalence provides flexibility, but also complicates motor planning.

When subjects were asked to select a single movement configuration from those available in the motor equivalence set.

Previous studies have shown that, at the end-point of aimed, arm movements, much of the inexactness associated with joint-level motor equivalence can be accounted for (Soechting and Flanders, 1985; Stains & Wright, 1993). Two factors may contribute to the noisiness of geometric constraints imposed by a fixed linkage system, and behavioral strategies. We evaluate their probable contribution by investigating aimed, arm movements performed with two excess degrees of freedom.

Arm configurations were measured while five subjects made repeated pointing movements from 10 starting points to a target. Five targets were tested on separate days. Movement is permitted in a horizontal plane, using four degrees of rotational freedom: one each at the right wrist, elbow and shoulder, and one at the torso.

We compared the movements sampled at the end-point of movements to a single target with the variability that would exist if all configurations within the motor equivalence set were sampled. Results show that behavioral strategies make a substantial contribution for all subjects, even though individuals' strategies differ dramatically. Effects of particular strategies are explored through simulation modeling.

Results suggest new techniques for evaluating models of joint-level motor equivalence (Rosenbaum et al., in press, Bullock & Greenspan, 1993, Bower & Cruse, 1990) by testing the extent to which they replicate the partitioning of variance between geometric and behavioral strategies observed here.
576.13 UNCONTRAINED SINGLE-JOINT MOVEMENTS IN INDIVIDUALS WITH DOWNS SYNDROME: G. L. Almeida*, L.-M. Hong*, M. D. Corcos, G. L. Costill*, *Rush Medical Center, Chicago, IL 60612, *University of Illinois at Chicago, Chicago, IL 60608 and *Universidade Estadual de Campinas, SP, Brazil.

Three individuals with Down syndrome performed unconstrained single-joint elbow and shoulder flexion movements, in a sagittal plane. First, the subjects were instructed to move one joint (the "focal joint") as fast as possible, while keeping the "non-focal" joint stationary. These movements were performed over three different distances. Second, the subjects were instructed to move a "focal joint" at three different speeds, over one distance.

The movements of the focal joint were accompanied by varying degrees of movements at the non-focal joint. On average, the movements at the non-focal joint were twice as large as those observed in neurologically normal individuals, and they displayed slower movement speed at the focal joint. For the movements performed over three distance, the pattern of muscle activity was typical for "speed insensitive strategy." The duration of the agonist EMG burst scaled with distance while their rate of rise remained constant. Nevertheless, there was more variability related to the antagonist latency. One subject presented a pattern of co-contraction, whereas for the other, the antagonist latency did not scale with distance.

For the movements performed at three different speeds, the pattern of muscle activity was typical of "speed sensitive strategy." The rate of rise of the agonist EMG burst scaled with distance with the antagonist latency being activated early for fast movements.

These findings suggest that, in comparison with neurologically normal individuals, individuals with Down syndrome use similar rules to control the muscle activation. Nevertheless, they seem to have a coordination problem which is reflected in their coupling of the muscle activity burst and in the large range of the movements in the "non-focal joint".

This work was supported by NIH grants NS 01505, AR 31849.

576.15 ANALYSIS OF KINEMATIC AND DYNAMIC PARAMETERS OF HUMAN MOTOR LEARNING: S.J. Staubes* and T.J. Elber, Departments of Neurosurgery and Physiology, and Graduate Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455.

In order to more fully understand the characteristics of human motor learning, human subjects were evaluated using a step-movement task. Subjects were seated in front of a video display and controlled the movement on the display with the right hand using a planar 2-joint manipulandum. A string supporting the elbow constrained movement to the horizontal plane to permit explicit determination of shoulder and elbow joint angles and torques. The task consisted of maintaining the cursor in a central "start box" until one of eight equidistant "target" boxes appeared; the cursor then had to be moved to and held in the target box within a movement time window. After practicing on a normal hand-cursor movement relationship, the gain was changed, forcing the subjects to learn a new relationship between hand and cursor movement. Once the subjects' performance had returned to an acceptable level, the gain was restored to the initial conditions. Initially, subjects' hand paths were nominally straight with characteristic bell-shaped hand velocity profiles and biphase accelerations. Biphase (accelerating) and "decorrelating" torque profiles varied predictably with direction during the normal gain condition. Upon initial presentation with the new gain, movement errors increased, with larger errors in directions involving larger shoulder torques, and smaller errors in directions involving larger elbow torques. Compared to other targets, learning in these directions appeared to be more difficult. The "accelerating" phases of the joint torques followed a stereotyped profile, while the "breaking" phases of the joint torques were prolonged in the early stages of learning, regaining their characteristic form as net performance improved. The correlation of the shoulder/elbow torques with the spatial dependence of the error suggests that the control scheme for reorganization of two-joint movements is more complex than that for single-joint movements.

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CIRCUITRY AND PATTERN GENERATION III


Rhythmic motor patterns of the dorsal ventricular nerve of the stomatogastric ganglion were monitored with chronically implanted electrodes in intact crayfish, *Cragon saxonus*, while the activity of the gastric mill and the cardio-pyloric valve was monitored by endoscopic imaging. Feeding experiments of free-moving crayfish demonstrate a decrease in the pyloric activity during presentation and grabbing of food which is followed by a strong increase in the pyloric frequency. After food intake the gastric activity starts vigorously and stays on a high frequency level for long duration (see figure). The activity level increases further depending on the amount of food.

pyloric frequency [s⁻¹]

food presentation

0.12

0.06

gastric frequency [s⁻¹]

0.5

1.0

40 s

Application of juice from mussels or fish into the stomach induces an amplification of movements of the gastric mill as well as a modification in the opening and closing of the cardio-pyloric valve. Gastric movements often show pyloric modifications which reflect complex gastric-pyloric patterns probably due to changes in the consistency of food. The investigations on the function of the stomatogastric system and neuroanatomical studies of the networks indicate the importance of sensorimotor mechanisms of integration which are involved in initiation and feedback control of the stomach's rhythmic motor-patterns. Supported by HFPSP.


It has been shown (Shadmehr and Mussa-Ivaldi, J. Neurosci., in press) that humans can adapt to virtual dynamic environments. We are interested in finding the neural mechanisms underlying this phenomenon. As a first step, before beginning the recording of single cells from cortical areas, it is necessary to assess how well the data observed in human subjects apply to macaque monkeys. Psychophysical tests were performed in an awake behaving monkey using the following paradigm: The monkey was trained to move a manipulandum in a set of targets sequentially appearing on a computer screen and hold the cursor in the target for at least one second in order to get a reward. In the baseline condition, the manipulandum behaves like a damper in order to help the monkey move smoothly. In the perturbation condition, non-homogeneous viscous field is superimposed on the baseline field. In the latter condition, a dramatic alteration of the trajectory was observed initially, followed by a gradual return to the baseline trajectory. This is consistent with the hypothesis of an underlying kinematic planner cascaded with an execution module canceling out the dynamics of the environment by learning a model of the disturbing field. We ruled out the possibility of co-contracting, which would be an alternative explanation of the data, by looking at the after-effects obtained by restoring the baseline condition. The after-effect appeared as a distortion in the path opposite to that induced by the field. Such after-effects decay rapidly when the monkey is longer exposed to the field. Acknowledgements: NIH grants N02043 and AR028710, and ONR grant N00014K0372. FG was supported by a fellowship by SSSA.

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577.3 NOVEL DEPOLARIZING GLUTAMATERIC CONDUCTANCE EXPRESSED IN CRUSTACEAN STOMATOGASTRIC MOTOR NEURONS IN PRIMARY CULTURE. T.A. Cleland* and A.L. Selverston, Dept. of Biology 0157, Univ. Calif. San Diego, La Jolla, CA 92039-0357.

The stomatogastric ganglion (STG) of the Pacific spiny lobster, Palinurus interruptus, is one of the most well-defined central pattern generators studied at the intercellular and circuit level. In this study, STG neurons were individually isolated in primary culture and tested in two-electrode voltage clamp. Calcium-dependent properties under improved space-clamp conditions and without the complicating effects of other networked cells. Glutamate-induced responses were characterized using two-dimensional phase-portraits. Biophysical and pharmacological characterizations of this glutamatergic conductance are presented.

Supported by NIH grant P01NS25916 to AIS and an NSF Predoctoral Fellowship to TAC.

577.4 MODES OF OSCILLATION AND SETTING RELATIVE PHASE IN SMALL MOTOR PATTERN GENERATING NETWORKS. P.E. Burr* and A.L. Selverston, Biology Department, U.C. San Diego, La Jolla, CA 92039-0357.

We have constructed a simplifying, biologically constrained, network model of the gastric mill CPG which shows that only a few basic mechanisms are sufficient to produce the relatively complex patterns characteristic of the gastric mill. These mechanisms include a cell model with a fast current with an N-shaped I-V curve and slow inward and outward currents with linear steady-state I-V curves; graded synaptic transmission; and "slow" synapses. The cell model captures important characteristic behaviors of gastric mill cells, such as plateau potentials, post-inhibitory rebound, and endogenous oscillation. The reciprocal inhibitory pair is an essential sub-network of the gastric mill network, which enables the cell model to produce a pattern whether or not the individual cells are endogenous oscillators.

An isolated cell has six distinct behaviors: endogenous oscillations; silent; almost an oscillator; plateau potentials; tonically firing, hyperpolarized. Using pairs of two-dimensional phase-portraits, we have studied in detail the modes of oscillation of two cells connected with reciprocal inhibitory synapses, in which the center of the synaptically transfer function, or "threshold", was allowed to be different for the two synapses. Every combination of behaviors in the individual cells can give rise to oscillations, provided the thresholds and weights are correctly adjusted. In the symmetric case, the cells are exactly out of phase, but when asymmetries are allowed many other phase relationships are obtained. In addition, one can get 1 N (N=1,2,3) or even M N phase locking, and chaos from the reciprocal inhibitory pair.

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The 8 py motoneurons in the stomatogastric ganglion of the spiny lobster Palinurus interruptus are integral members of the pyloric central pattern generator and innervate 13 intrinsic constrictor muscles on the pylorus. Electrophysiological differences between cells of this class have been acknowledged for many years (Hartline, et al., 1987). Consistent with previous studies we found two distinct physiological phenotypes which can be quickly identified. PY cells do not respond to stimulation of the hepaticorenal duct nerve (Hdnl) or to a bell (10-4 M) applied on the abdomen (DA), are strongly electrically coupled to LE cells (-5 mV into LE cell), are strongly active during pyloric cycling, and can overlap the LP firing phase in a combined preparation. PY cells do respond to 5-HT, respond weakly or not at all to DA, and are not detectably electrically coupled to LP(-5 mV into PY); they are often silent during the ongoing pyloric rhythm and never overlap LP activity in a combined preparation. Four superficial muscles (p2,8,10,12) were used to test for differences in innervation patterns between the PY subtypes. The results showed no segregation of innervation on the muscles by other subtype; each muscle is innervated by at least one neuron of each type. We are currently looking at PT heterogeneity at the level of expression of mRNA for the skater K+ channel family using in situ hybridization. Supported by NIH NS17523 and SPOI NS25915.

577.6 EFFECT OF DOPAMINE ON THE TRANSIENT POTASSIUM CURRENT AND THE HYPERPOLARIZATION-ACTIVATED CURRENT IN PY AND LP NEURONS OF THE PYLOCIRIC MOTOR CIRCUIT. L.M. Congiglio and R.M. Harris-Warrick, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

The stomatogastric ganglion of the lobster, contains a network of neurons, called the pyloric network, which controls rhythmic movements of the pylorus, part of the lobster foregut. The pyloric motor pattern is altered by the endogenous neuromodulator dopamine. The effect of dopamine on the pyloric pattern is due to both synaptic connections between neurons of the pyloric network and direct effects on the neurons themselves. We show that in two cell types, PY cells and LP cells, dopamine specifically affects the transient potassium current, Ih. In the PY cell type, dopamine decreases Ih by reducing its maximum conductance, shifting its voltage of half-activation in the depolarized direction, and increasing its rate of inactivation. In the LP cell, dopamine also decreases Ih by reducing its maximum conductance and shifting its voltage of half-activation in the depolarized direction. In the LP cell, however, dopamine has no effect in the rate of inactivation of Ih. In addition, in the LP cell dopamine affects a second current, the hyperpolarization activated current Ih. Dopamine effectively increases Ih by shifting its voltage-dependence of activation in the depolarizing direction and increasing its rate of activation.

Understanding the specific effects of dopamine on these neurons is important in understanding how alterations in particular neuronal properties can underlie the alteration of a motor pattern.

Supported by NIH grant NS17523.

577.7 DISTRIBUTED ANINE MODULATION OF GRADED CHEMICAL SYNAPTIC INTERACTIONS BETWEEN NEURONS OF THE PYLOCIRIC MOTOR NETWORK. R.R. Johnson*, L. Peck and R.M. Harris-Warrick, Section of Neurobiology and Behavior, Cornell University and Dept. of Psychology, Ithaca College, Ithaca, NY.

Dopamine (DA), seratonin (5HT) and octopamine (OA) are endogenous neuromodulators in Crustacea, and each evokes a distinct motor pattern from the quiescent pyloric network in the stomatogastric ganglion of the spiny lobster. We examined amine effects on graded chemical synaptic transmission between known synaptically isolated pairs of neurons in the pyloric network to determine if these could contribute to the amine-generated motor patterns. Each amine had a unique and distinct spectrum of transmitter effects on graded chemical synaptic transmission across the pyloric synapses. DA (10-6 M) enhanced some pairs of synaptic interactions and weakened others. 5HT (10-6 M) also enhanced and weakened different pairs of synaptic interactions, but to a lesser degree than DA. OA (10-6 M) strengthened some synaptic interactions and had no effect on others. At some previously described chemical synapses, amine effects were comparable to those reported for octopamine (OA). However, these neuromodulators are required for the functional expression of some chemical synapses within the pyloric network. This completes our survey of the functional role of several neurotransmitters in chemical and electrical synaptic interactions within the pyloric network. Supported by NIH grant NS 17523 and the Human Frontier Science Program.

577.8 FUNCTIONAL ROLE OF ELECTRICAL COUPLING IN CONTROLLING THE LOCAL ACTIVITY OF A MODULATORY PROJECTION NEURON. M.J. Coleman*, P. Menvie* and M.P. Huebna*, 1Department of Physiology and Biophysics, Univ. Alabama at Birmingham, B'ham, AL 35294/Dept. of Neuroscience, Univ. Pennsylvania Medical School, Philadelphia, PA 19104; 2Lab. de Neurobiologie et Physiologie Comparées, CNRS, 33120 Arcachon, France. Intracellular recordings at the entrance to the stomatogastric ganglion (STG) of the crab Cancer borealis show that the STG terminals of modulatory commissural neuron 1 (MCN1) excites the pyloric and gastric mill rhythms in the STG, receives IPSPS from the lateral gastric (LG) neurons, and is weakly-coupled to LG (Nusbaum et al., J Neurosci. 12:2708). The LG inhibition of MCN1 controls the gastric mill rhythm-terminating activity of MCN1 in the STG (Coleman & Huebna, J Neurosci. in press).

Despite the synaptic inhibition from LG to MCN1, LG can also influence MCN1 via the electrical coupling between these two neurons. For example, depolarizing LG to levels sufficient to elicit transmitter release activates MCN1. Slightly stronger LG depolarization first activates and then inhibits MCN1. When the LG-mediated inhibition is eliminated by bath-applying picrotoxin (10-5 M), LG stimulation activates MCN1 and later enables MCN1 to activate its STG targets.

MCN1 stimulates gastric mill rhythms, the MCN1 hyperpolarization is largest at the start of each LG burst. This response in MCN1 then exhibits a depolarizing sag, despite the fact that MCN1 is hyperpolarized due to its underlying membrane potential. This enables MCN1 to escape from inhibition before the end of the LG burst. We are studying whether the electrical coupling between LG and MCN1 contributes to this escape facilitation in MCN1.

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THURSDAY AM
MUSCARINIC MODULATION OF EXCITABILITY AND SYNAPTIC OUTPUT IN A GASTRIC PATTERN-GENERATING NEURON OF THE LOBSTER STOMATO garganglion. R.E. Skinner and A.I. Selverston, Department of Biology, UCSD, La Jolla CA 92039.

In the isolated stomatogastric ganglion of spiny lobsters, the muscarinic agonist, pilocarpine, activates gastric output neurons in the gastric mill CPG. The lateral gastric neuron, LG, an important pattern-generating cell, goes from quiescence to spontaneous spiking and then to firing strong bursts underlaid by plateau potentials.

Even before the onset of spontaneous activity, however, one can detect a large increase in the amplitude of LG's slow afterhyperpolarizing potentials (SAPs) to other gastric neurons. IPSs to PDs are evoked by ortho- and antidromic spikes grow larger, the input-output function for graded synaptic transmission gets steeper, and the threshold for transmitter release is seemingly reduced when concurrent coupling is monitored neurally input resistance during synaptic potentiation, and found a large increase in synaptic conducance. The resting input resistance of LG could also increase, but is not clear is LG conducts all for the all-observed potentiation.

Currently, pilocarpine decreases LG's spike threshold and increases the slope of the spike frequency action potential. Together these changes enhance the role of spike-mediated transmission at low presynaptic depletions. Also induced is a slow, seemingly active, depolarization, which may be the plateau current, and which is a good driver of graded transmission.

A possible interpretation of these results is that munc43 modulates lead to enhanced activation of calcium currents in LG's distal neurites, allowing greater transmitter release. Present experiments seek to identify which conductances are modulated in LG to and to clarify how these changes relate to modifications of excitability and efficacy. Supported by NIH grants NS0152916, NS09322 and PH507220.


We characterize how modulation of an intrinsic conductance (I) alters the responses of a half-center oscillator formed by reciprocally inhibitory connections. We use a combined theoretical/experimental approach employing conventional models and neuronal recordings, to examine the dependence of dynamic clamp (Sharp et al., J.Neurophys., 69-992,1993). A plot of network period vs. synaptic threshold produces an inverted U-shaped relationship (see accompanying abstract). As the maximal I conductance is increased the I conductance is organized as a cycle of activity. In the direction, the period vs. synaptic threshold relationship is shifted in the depolarizing direction. This indicates that these changes cause a frequency increase if a synaptic threshold is increased and a frequency decrease if a synaptic release mechanism is in operation. For a small range of synaptic threshold values, it is also possible for the system to switch between synaptic escape and release modes for these changes. If the maximal conductance is made too small or the activation curve is shifted too far in the hyperpolarizing direction, oscillations cease. This indicates that there is a critical relationship and intrinsic properties in order to generate sustained half-center oscillations. This differs for a detailed understanding of these relationships. Supported by NIMH MH57472 and NSF BNS0090251.

RECIROCALLY INHIBITORY NEURAL NETWORKS: EFFECTS OF VARIATIONS IN SYNAPTIC PRINCIPLES. F.K. Skinner, A.A. Sharp, and E. Marder. C. for Complex Systems and Dept. of Biology, Brandeis University, Waltham MA 02254-9110.

Reciprocally inhibitory circuits are ubiquitous components of oscillatory neural systems. We characterize how modulation of synaptic properties alter the responses of such a two cell system (half-center oscillator) using a combined theoretical/experimental approach. We employ two two-compartment models. This includes: a) model in which includes HH-like spikes. Experimentally, we take advantage of the dynamic clamp technique (Sharp et al., J. Neurophysiol., 69-992,1993) to couple pairs of gastric mill neurons in the stomatogastric ganglion, and to introduce artificial I. As the synaptic threshold is increased, the frequency decreases and then increases. This frequency transition occurs as the system switches from a synaptic escape mechanism to a synaptic conductance mechanism (Skinner et al., J. Computational Neurosci., in press). As the synaptic time constant is increased, the network frequency and oscillator amplitude both decrease. Increasing the maximal synaptic conductance decreases a frequency decrease and an increase in oscillator amplitude. For large synaptic conductances, oscillations can not be initiated for values of the synaptic threshold which fall in the transition between synaptic escape and release mechanisms. This interactive approach allows for a detailed understanding of how interactions between synaptic and intrinsic parameters generate network behavior. Supported by NIMH MH46742 and NSF BNS0090251.


The pyloric network neurons types are distinctly different on the basis of muscle innervation, electrophysiological activity, and synaptic connectivity in the network. We examined whether the individual neuron types also have different morphologies by injecting neurons with the fluorescent dyes Lucifer yellow or fluorescein dextran and reconstructing the neurons with a Eustacic three dimensional neuron tracing system.

In agreement with the results of King and Selverston (1976) and Selverston and Mullenby (1973), the main neurite of the Ventral Terminal (V) neuron splits in the neuropil into two processes, each of which continues to form one of the neuropil axons. In addition, within the neuropil, the LG neuron distinctly has a process that leaves the main neurite at or before the axon initiation zone and then goes around the neuropil. Detailed analysis of the neuropil neuron of the two axonal processes reveals they occupy non-overlapping regions of the neuropil.

The pyloric network has two Pyloric Dilator (PD) neurons that seem to be identical electrophysiologically. However, these neurons appear to have two different morphologies. In one class (n=4), the main process loops over itself as it continues to run in the neuropil. The other PD neurons have less ramifications than the former class (n=7) and have more distant initial branches (av. distance from soma, 133±41nm). In four PD neuron fills, one of each class was present in the ganglion. In a fifth very poor double fill, I could see connective tissue that filled up with PD neuron profiles were present.

These results suggest that at least some the pyloric neuron types have distinct morphologies. Perhaps more interestingly, the distinct volumes occupied by the PD neuron neurite processes could provide for a basis for anatomical segregation of the stomatogastric neuropil.


The two pairs of dorsal dilator muscles are an invertebrate also innervated by the Pyloric Dilator (PD) neurons and have been assayed to express a pyloric motor pattern. We examined the intrinsic constrictions of these muscles in vitro preparations maintained in oxygenated canine saline solution. In 3 of 5 preparations, we observed no or only very small (<0.1mm) pyloric timed contractions. In the other preparations these large (~1mm), long duration (4s) contractions 6 to 8 sec, very different from the PD neuron burst length (~250ms) and cycle period (~1s) present in these preparations. In 10-4M dopamine (DA), all preparations large to (up to 2mm) contractions, with long durations and periods. Large (>0.1mm) pyloric timed activity was often concentric in either the DA or VP.

In two experiments, we observed that the large contractions were phase locked with Gastric Mill (GM) neuronal activity, and that constriction duration covered with GM neuron burst length. In one experiment we stimulated the anterior lateral (aln) nerve, which contains GM neurons axons; elicited, at constant short latency, contractions whose duration was matched with the stimuli duration. In the other, we stimulated the posterior lateral (pln) innervated by the pyloric muscle, the large contractions were much longer than the pyloric period, suggesting that even if pyloric activity is present, the pyloric escape mechanism in either the DA or VP. This idea was tested by applying DA to augment contraction amplitude and stimulating the lateral ventricular (IVN) nerve or aln using parameters (40Hz for 250ms every 1s) mimicking pyloric neuron bursts. Under these conditions the contractions summed, resulting in a sustained contraction.

These results suggest that the dorsal dilator muscles, at least under some conditions, 1) may respond to pyloric input with sustained contraction and 2) can express gastric mill timed and duration motor output.

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The rhythmic pattern produced by the lobster pyloric network maintains relatively constant phasing as cycle frequency is altered by current injection into the pyloric pacemaker. Perfect phase maintenance requires appropriate changes in both pacemaker burst duration and firing delay of follower neurons after injection. Previously I showed that when trains of hyperpolarizing current pulses are injected into isolated pyloric neurons, firing delay of Inferior Cardiac (IC) and Lateral Pyloric (LP) neurons primarily depends on pulse amplitude, whereas Pyloric (PY) neuron firing delay primarily depends on the temporal characteristics of the input current.

Intracellular recordings from LP and IC neurons in the intact network show that injection of current pulses into these cells changes as pyloric frequency is altered. However, this change is small and opposite to the change expected if the cellular properties of these neurons play a role in phase control. Re-analysis of prior experiments with respect to delay after the pacemaker burst, rather than phase, shows these neurons fire with a constant lag after pacemaker activity.

In the intact network, PY neurons do show appropriate changes in firing delay, and thus the firing delay properties described previously likely are relevant to their phasing. This work did not directly address whether PY neuron firing delay depends on pulse length or interpulse interval. Experiments in which these lengths were varied independently showed that inter-pulse interval, not pulse length, mainly determines PY neuron firing delay.

Maintenance of LP and IC neuron phase as pyloric frequency changes thus is largely due to the associated changes in pacemaker burst length. PY neuron phase maintenance, alternatively, is likely associated with PY neuron cellular properties that alter neuron firing delay as pyloric frequency is altered. However, this delay may be primarily determined by the length of the PY neuron burst in the preceding pyloric cycle, not the changing duration of pacemaker inhibition received by the PY neuron.

ASSOCIATION CORTEX AND THALAMOCORTICAL RELATIONS

MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL PROPERTIES OF LAYER 1 NEURONS IN RAT NEOCORTEX. F.M. Zhou* and J.J. Habibz, Neurobiology Research Center, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

The basic morphological properties of layer 1 neurons were described by Ramon y Cajal about 100 years ago. Yet, the electrophysiological properties of these neurons has remained an enigma and their neuronal nature has been questioned. Using an in vitro slice preparation, we have examined the morphological and electrophysiological properties of visually identified layer 1 neurons. Whole cell patch clamp techniques were used in slices from 2-3 week-old rats. Biocyn labeled cells were multipolar with somas 10-20 μm in diameter. Most cell processes were smooth and spread horizontally for 1-2 mm within layer I. Some presumed axonal processes going to layers II-IV were observed. All the layer 1 cells (N=30) tested had electrophysiological properties typical of interneurons. Action potentials evoked by depolarizing current pulses showed minimal or no frequency adaptation. Action potential durations, measured at the base, were short compared to pyramidal neurons recorded under similar conditions (2.5-5 ms at 22°C). In the presence of 20 μM APV and 10 μM CNQX, bicuculline-sensitive spontaneous IPSCs were recorded in the majority of cells. The amplitude of sIPSCs ranged from less than 10 pA to more than 200 pA, at a holding potential -60 mV in symmetrical Cl-, and reversed at 0 mV. Adding 0.5 mM TTX reduced sIPSC frequency and blocked large amplitude sIPSCs. These results suggest that layer 1 neurons have firing properties characteristic of interneurons and receive inhibitory inputs. They appear to have extensive processes which may serve in controlling cortical excitability. (Supported by NS18145 and NS22373)

TWO DISTINCT PATHWAYS FOR ACTIVATING cGMP IN THE CRAB STOMATOGASTRIC NERVOUS SYSTEM. K. L. Schütz, M. F. Guy*, A. J. W. Trammell, and K. Grabaush*, Univ. of Washington, Dept. of Zoology, Seattle, WA 98195 and Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27599.

We are investigating pathways of cGMP activation in the crab stomatogastric nervous system (STNS), a collection of motor circuits known to be extensively modulated by both synaptically-delivered transmitters and circulating neurohormones. Using antisera selective for cGMP, we have conducted parallel radioimmunoassay (RIA) and immunocytochemical (ICC) studies to screen for stimuli that activate cGMP synthesis in the STNS. We have found two classes of activators that are effective; nitric oxide (NO) donors and peptide-containing extracts of crab sinus gland.

In RIA studies, three NO donors (SNP, SNAP, and SIN-1) were all found to produce large increases in cGMP levels when applied with the phosphodiesterase inhibitor IBMX. A similar result was obtained with ICC studies; treatments with NO donors and IBMX consistently caused the appearance of cGMP immunoreactivity in a subset of neurons. We used an arginine-to-citrulline conversion assay to screen tissues in the vicinity of the STNS for a nitric oxide synthase (NOS), and found a candidate in crab heart. This tissue contains an arginine-utilizing enzyme with properties that resemble a constitutive NOS, including calcium-dependence, NADPH-dependence and sensitivity to arginine analogs. No such activity was observed in the STNS, the crustacean digestive system. Neurons that contained the cGMP content of the STNS when applied with IBMX. We are currently using cGMP ICC to identify which cells are targeted by the active component of the extract; preliminary results indicate that the peptide extract has a different target specificity than do the NO donors. Based on these results, we suggest that NO and a sinus gland peptide represent two non-overlapping pathways for activating cGMP in the STNS. Supported by an NRSA fellowship to N.S., NIH grants (NS15697 to K.O. and NS 25915 to M.G.) and NSF grant IBN-9245993 to J.T.

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578.3 Efferent projections from the rostral thalamic reticular nucleus to the ipsilateral and contralateral thalami: An ANTONERI and POSTERIOR techniques in the monkey. R.D. Burwell, M. Caballero-Bleda, M. Guillery, and D.G. Amaral.1 Department of Neurophysiology, Neural Institute “C. Besta,” 20133 Milano, Italy.

The conventional view that the intralaminar connections are sparse or absent has been recently challenged by anatomical studies from different groups (Bostiglio et al., 1995; Petre and Sorine, 1995; Boultham et al., 1994). These studies, mainly based on anterograde or retrograde tracer experiments, have been performed in the macaque by injecting the thalamic reticular nucleus (TRN), and particularly its rostral part, to contralateral (as well as to ipsilateral) thalamic nuclei. The retrograde transport of biotin- derived amine (BDA) was employed in the present study to investigate the connectivity of the rostral TRN and the medial, ventral, and lateral regions of both hemispheres. The present study was designed to test whether projections from the rostral thalamic nuclei of the contralateral hemisphere provide a mechanism for the interhemispheric transfer of information.

After BDA injections in the medial region of the contralateral TRN, anterogradely labeled fibers were observed in the corresponding regions of the ipsilateral thalamus. The present study demonstrates the thalamic network involved in interhemispheric connectivity and the role of the rostral part of the TRN in reciprocal connections of the thalamus. The present data suggest a reciprocal connectivity through the thalamic network and the role of the rostral TRN in reciprocal connections of the thalamus. The present data demonstrate the importance of the rostral TRN in reciprocal connections of the thalamus and the role of the rostral TRN in reciprocal connections of the thalamus.

578.5 A PHA-L STUDY OF THE PERIRHINAL PROJECTION TO THE THALAMUS IN THE RAT. R.D. Burwell,1 M. Caballero-Bleda, M. Witter,2 and D.G. Amaral.31 The Center for Behavioral Neuroscience, SUNY at Stony Brook, Stony Brook, NY, 11794-3755.2 Anatomy and Neurophysiology, Vrije University, Amsterdam, The Netherlands.

The precise relationship of certain thalamic nuclei with distinct regions of the neocortex has provided one mechanism for defining the cytoarchitectonic boundaries of the cortical mantle. This study was designed to determine whether projections from various locations of the perirhinal cortex might differentiate subdivisions of this region. Using adult male Sprague-Dawley rats as subjects, the anterograde tracer, Fluoro-gold (FG), was injected into the lateral entorhinal cortex. After a survival period of 7 days, brain sections were stained with FG to test for the presence of labeling.

Cross-sectional studies in this region revealed a pattern of labeling that was consistent with the pattern observed in the monkey. The perirhinal cortex is characterized by a dense distribution of FG-labeled neurons that is consistent with the pattern observed in the monkey and consistent with the pattern observed in the monkey.

578.6 INTRINSIC CIRCUITS OF FUNCTIONALLY SPECIALIZED SUBDIVISIONS OF HUMAN CEREBRAL CORTEX. M.F. Krubitzer. Department of Neurology and Behavior, SUNY at Stony Brook, Stony Brook, NY, 11794-2520.

The present study was designed to determine whether connections from various subdivisions of the neocortex might differentiate subdivisions of this region. Using adult male Sprague-Dawley rats as subjects, the anterograde tracer, Fluoro-gold (FG), was injected into the lateral entorhinal cortex. After a survival period of 7 days, brain sections were stained with FG to test for the presence of labeling.

Cross-sectional studies in this region revealed a pattern of labeling that was consistent with the pattern observed in the monkey. The perirhinal cortex is characterized by a dense distribution of FG-labeled neurons that is consistent with the pattern observed in the monkey and consistent with the pattern observed in the monkey.

578.7 EVOLUTION OF THE FRONTAL LOBES: AN MRI STUDY ON APES AND HUMANS. K. Smedes,2 H. Damos,2 and G.W. Van Hoosier3.3 Dept. of Anthropology,1 Neurology2 and Anatomy3, Univ. of Iowa, Iowa City, IA 52240.

How much did the frontal lobes (FL) enlarge during hominoid evolution and which of the subdivisions (dorsal, mesial, orbital) changed the most? We studied 5 living hominoids: chimpanzees, gorillas, orangutans, and a monkey (macaque). Thin cut MRIs were obtained from non-human primate brains and from a human living subject. All were reconstructed in 3-D. Major landmarks were determined and used to generate the coordinates of a dorsal, mesial and orbital region of interest in each hemisphere. The overall size of the hemisphere was compared with CT a second arctan and white matter and subcortical grey structures.

Relative to the rest of the hemisphere, the size of the FL is largest in the chimpanzee, where it is nearly identical (36.3% and 36.9% of the same hemisphere). The area and gyri have the smallest (31.3% and 31.5%) (1). The FL show a trend towards increased size in hominoid evolution, but the 3 sectors did not change much. Across the three species, the rostral and orbital sectors are more, but not, in a relative sense and, as expected, the dorsal sector is largest followed by the mesial and orbital. A noteworthy difference was found in the fourth sector, the frontal lobe, where the human frontotemporal region is largest. The modest marked difference was seen in the anterior third, which contains the white matter, underlying prefrontal cortices.

Support from the National Institute of Health, grant RO1 19632-11. We thank Yerkes Regional Primate Research Center, Bush Gardens Zoo, Houston Zoo, Toledo Zoo, Gladys Porter Zoo and Metro Washington Park Zoo for providing the ape specimens.

578.8 SYNAPTIC PHYSIOLOGY OF THE AMYGDALA-PERIRHINAL PATHWAY STUDIED IN VITRO. J.M. Szego.1 and J.W. Kaftans. Department of Physiology and Center for Neuroscience, Yale University, New Haven, CT 06511.

Although cortical and limbic areas have been suggested to play complementry roles in mammalian memory, little work has been done to study synaptic interactions between these two regions. To address this issue, we have performed experiments in vitro in an in vitro slice preparation which maintains connectivity between perirhinal cortex (PRh) and the lateral nucleus of the amygdala (LA). Here we describe experiments using the optic fiber technique for reliable preparation of these slices and present the results of studies of synaptic plasticity in this system.

Coronal slices 400µm thick were obtained from brain in the range of -2.2 to -3.8 from brain. Stimulation with current pulses of 50-100µA in PRh in 33% of these slices. Current-source density analysis reveals a current sink restricted to layers III-IV of PRh. Injection of the lipophilic tracer DiI also confirmed that a fiber pathway with contralateral labeling in the LA is present in a subfield of the R. LA. These data indicate that there is a component that can follow at 100Hz with a constant latency of 5ms over a range of stimulation intensities of 0.2-2Apep.

To examine use-dependent plasticity, field potentials were recorded in superficial layers of PRh while applying high-frequency stimulation in LA. Preliminary results indicate that these responses can be potentiated. Stimulation applied to superficial layers of adjacent cortical areas can either potentiate or inhibit these responses recorded in a superficial PRh. We are investigating whether there exists cooperativity or associativity between the LA and intracortical inputs to PRh. Future studies will examine whether this plasticity is NMDA-dependent, and if the input from LA can serve as a "reinforcement signal" to consolidate intracortical plasticity in PRh (Supported by NIH and Yale University).

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758.9

The excitatory axon terminals that furnish intra- and inter-areal connections in monkey prefrontal cortex (PFC) are both arranged in discrete clusters. Although, little is known about the organization of these two types of terminal fields, it seems certain that the distribution of their origins of origin. Single injections (150 μl diam.) into sectioned tissue of areas 9 or 46 of microwave monkey PFC produced two distinct types (intra- and inter-areal) of clusters of immunohistochemically characterized axon terminals. Within areas 9 or 46, labeled terminals formed multiple stripes (45 × 1.4 μm) in layers 1-3 that were similar to the intraregional lattice structure formed labels in the medioapical collateral of layer 2/3 presumed as revealed by the tracer biocytin (Levit et al., 1993). Terminals from the same injection also formed bands (49 × 1.2 mm) at substantially greater distances from injection site. These axon terminals, which were distributed across all cortical layers, arose from labeled axons in the underlying white matter. Similar injection of cholera toxin B (CTB) revealed two analogous types of clusters of retrogradely-labeled pyramidal neurons. Within areas 9 and 46, labeled neurons formed stripes (42 ± 1.2 mm) primarily in layers 2/3, whereas in other PFC regions, clusters of labeled neurons were present in both superficial and deep layers. Anterogradely-labeled CTB axon terminal at both the intra- and interareal cell clusters, revealing the reciprocity of these connections. These findings confirm the discontinuous distribution of both intra- and interareal connections in monkey PFC. They also demonstrate that each point in the intrinsic lattice furnishes divergent output to multiple zones within the lattice as well as to other PFC regions, and they converge input from these same zones. These patterns of reciprocal intra- and interareal connections provide an anatomical substrate for the simultaneous and recurrent activation of specific distributed networks of neurons in monkey PFC.

758.10

Pyramidal neurons in the superficial layers of monkey prefrontal cortex (PFC) furnish horizontally spreading axon branches that form the same type of lattice comprised of multiple stripes (Levit et al., 1993). Because the function of the intra-areal circuitry depends on the input and output of the cells which furnish it, we investigated the dendritic morphology and extrinsic projections of these neurons. Small iontophoretic injections of biotinylated dextran amine (BDA) were made into areas 9 or 46 of monkey PFC. Retrogradely-labeled neurons 1) which were labeled via their axonal collateral projections, and 2) which BDA provided complete filling of dendrites. Typical features of these pyramidal neurons included: location in layers 2/3, an axon which emitted collaterals to the cortical surface at a shallow dendrites extending into layer 1, and basal dendrites which were confined to layers 2/3. The horizontal extent of the dendritic arbor (250-450 μm) was similar to the width of intrinsic neuronal lattice (Levit et al., 1993), suggesting that these neurons sample input from a single stripe. In order to determine the destination of the extrinsic projections of these neurons, dual injections were: 1) cholera toxin B (CTB) in areas 9 or 46, to label neurons with intrinsic axon collaterals within these areas, and 2) Fast Blue (FB) in another area of PFC, to label association-projecting neurons. In locations where the axons overlapped, 40.6% (54/135) of FB-labeled neurons were also labeled with CTB. Thus, neurons which furnish axonal collaterals of the intrinsic lattice also provide association projections. The lamina location of the dendritic arbor of these neurons indicates that they may receive inputs and/or project to other cortical areas. These features suggest that the intrinsic lattice plays an important role in cortical information processing by permitting the simultaneous activation of discrete, spatially segregated groups of neurons, resulting in a specific pattern of output from the PFC to other cortical areas.

758.11
DENDRITIC MORPHOLOGY OF NEURONS WITH DIFFERENT AXONAL PROJECTIONS IN MONKEY PREFRONTAL CORTEX. A.S. Sologyai*, M.L. Popack, and D.A. Lewis.1,2. Deps. of Neuroscience1 and Psychology1, Univ. of Pittsburgh, Pittsburgh, PA 15260.

The dendritic morphology of pyramidal neurons, the major source of excitatory intra- and interareal cortical connections, has been reported to differ depending upon the target of their effector axons. In addition, separate populations of neurons have been shown to furnish associated and collateral projections from a given region of monkey prefrontal cortex (PFC). In this study, injections of the fluorescent tracer Fast Blue (FB) into areas 9 or 46 of cynomolgus monkey PFC were used to identify associative and collateral neurons in these areas. Retrogradely-labeled neurons were then intracellularly injected with Lucifer Yellow in fixed slices (300 μm), converted to an immunoperoxidase label, and reconstructed using the Magnetic Neuron Tracing System. Neurons providing associative projections were pyramidal or modified pyramidal cells located predominantly in layers 2/3, with a smaller number in layers 5/6. Association neurons in the superficial layers shared the following characteristics: an apical dendrite which extended into layer 1, a basilar dendrite which was confined to layers 2-3, and a dendritic arbor with a horizontal spread of 200-400 μm. The laminar location, vertical and horizontal spread of the dendritic tree, and the pattern of dendritic arborization of association neurons is quite similar to that of neurons whose axon collaterals form the lattice-like structure of intraareal connections. These similarities are consistent with other evidence indicating that a subpopulation of PFC neurons furnishes both intra- and interareal connections (Michelzky et al. 94). Collateral neurons were also similar to association neurons in their laminar distribution and soma morphology; quantitative assessments of dendritic arborization and complexity, as well as measures of excitatory input, will be used to directly compare these two non-overlapping cell populations.

758.12

The calcium-binding protein, calretinin (CR), is present in a morphologically distinct population of local circuit neurons in superficial layers of monkey prefrontal cortex (PFC). This morphological cell class has previously been targeted to both pyramidal and non-pyramidal neurons. We examined the ultrastructural features of these neurons in areas 9 and 46 of monkey PFC using preembedding immunogold marker and investigated their association with DA terminals by combining immunogold staining with peroxidase immunoelectron microscopy for tyrosine hydroxylase (TH) and a postembedding approach to study synaptic contacts with cell soma, as well as large and small diameter dendrites. These contacts were primarily of the symmetric type. Many of the small dendritic targets had the morphological characteristics of typical pyramidal neurons; however, they were not immunoreactive for CR. CR immunoreactivity was also observed in small nonpyramidal neurons whose dendrites were distinctly varicose and received abundant synaptic input. In double labeled sections, immunoreactive for CR were frequently observed in adjacent areas of the neuropil. However, appositions between labeled processes were rare, and specific instances of synaptic input were not observed. Although our previous findings demonstrated that DA terminals directly innervate some GABAergic interneurons in the PFC, the present findings suggest that CR-containing interneurons are not included in this target population. However, a more indirect modulatory interaction between CR and TH-containing processes is suggested by their common distribution.
CORTICOCTORTAL CONNECTIONS MEDIATE DIRECTED ATTENTION IN RATS: BEHAVIORAL AND ANATOMICAL EVIDENCE. R.L. Reep*, J Vandevelde, E. Duckworth, M. Stoll and T.V. Corwin. Department of Physiological Sciences, University of Florida, Gainesville, FL 32610; and Department of Psychology, Northern Illinois University, DeKalb, IL 60115.

We investigated the role of corticocortical connections in directed attention by selectively disconnecting areas AGm and FPC, then testing animals for signs of hemispatial neglect. In rats, these two cortical areas are involved in spatial orientation and are highly interconnected via rostrocaudally directed axons traveling both directions in the deep gray matter. Therefore, coronally oriented knife cuts which extend into layer VI but do not erode upon the white matter should selectively transect these axons without interrupting those directed subcortically.

Knife cuts extending into layer VI (N=6) produced neglect, whereas control cuts extending only as deep as layer V (N=4) did not (p<0.02). The deeper knife cuts resulted in appreciably less axonal labeling than normal and a very sparsely labeled region rostral to the cut, whereas control subjects with shallower cuts exhibited labeling which was comparable to normals.

These results suggest that the corticocortical axons linking AGm and FPC play a pivotal role in directed attention.


Considerable progress has been made in recent years in determining the CNS mechanisms which mediate vomiting. However, the neuronal substrates that subserve nausea are completely unknown. The present study used functional neuroimaging techniques to determine if a localized cortical area is activated during nausea. Functional data were obtained using a 37-channel SQUID-based magnetic source imaging (MSI) device (Magnes, Biomagnetic Technologies Inc., San Diego, CA) and were overlaid on neuroanatomical information from high-resolution 3D SPGR MRI. Data were analyzed for the occurrence of transient bursts of neural activity exceeding 6000 fT when data were filtered in the range 8-100Hz). These were used to derive the location of the responsive neural source using the single equivalent dipole model. Data have been obtained from one volunteer in which nausea was produced on two separate days by either oral ingestion of syrup of ipecac or by visual stimulation (induced by head movements during yaw-axis rotation, while wearing vision-reversing goggles). Current dipoles indicative of neuronal activation were localized to a 2-3cm diameter region of cortex in the inferior frontal gyrus, when nausea was produced by either stimulus. Such activity was not observed during baseline controls or during sequences involving swallowing or exaggerated respiratory movements. While preliminary, the data suggest that MS1 may provide a quantitative means of measuring the effects of various pharmacological interventions on nausea and suggest a new CNS target for such intervention.

Supported by grants from NIH (NS20885 to AM).

APOMORPHINE ADMINISTRATION PRODUCES ACUTE DOSE-DEPENDENT RECOVERY FROM NEGLIGENCE FOLLOWING UNILATERAL POSTERIOR PARITIAL CORTEX LESIONS IN RATS. T.V. Corwin*, G.J. Altman, J.K. Bushman, and C. Goetz. Psychology Dept., Northern Illinois University, DeKalb, IL 60115

Previous research from our laboratory (Corwin et al., 1986; King and Corwin, 1990) has indicated that apomorphine (apo) has an acute dose-dependent therapeutic effect on neglect induced by unilateral lesion of the posterior parietal cortex (PPC), the present study examined the effects of apo administration on neglect induced by unilateral destruction of the PPC.

Subjects were 20 Long-Evans hooded male rats. After extensive handling, the subjects received an injection of either the left or right PPC via aspiration. At 48-96 hrs post-surgery, the subjects were tested for the presence of multimodal neglect, determined by the degree of head orientation in response to presentation of visual, tactile, or auditory stimuli. Subjects which met the criterion for severe neglect (defined by a ratio of responsiveness of the neglect side/non-neglect side of <1:3) were randomly assigned to one of four apo dosage groups: vehicle, 0.1, 0.3, or 0.5 mg/kg. Apo or the vehicle was administered i.p. 45-60 min after behavioral testing. Eighteen minutes post-injection, the subjects were retested. All behavioral testing was done with the experimenter "blind" with respect to the dosage administered.

There was a significant reduction in the severity of overall neglect in the 0.3 and 0.5 mg/kg groups (p<.05), but not in the vehicle or 0.1 mg/kg groups. The results indicated that apo produces an acute dose-dependent therapeutic effect on multimodal neglect produced by PPC lesions. As found for the AGm, neglect produced by unilateral PPC removal may be a result of disruption of dopaminergic mechanisms.

**COMPARATIVE NEUROANATOMY: SENSORY SYSTEMS**

SOME MORPHOLOGICAL FEATURES OF A VISUAL THALAMIC NUCLEUS IN A REPTILE. M.B. Fritz*, Dept. of Neurol. Surg., Indiana U. Sch. of Med., Indianapolis, IN 46202-5124.

Nucleus rotundus is a prominent nucleus in the dorsal thalamus of all non-avian vertebrates. In one group of reptiles, Catoblepis crocodilis, this neuronal aggregate contains no cells only and lacks local circuit neurons. The present study investigated some aspects of rotundal cytoarchitecture based on examination of Nissl morphology. Neurons were not uniformly distributed throughout the nucleus but were grouped in clusters commonly ranging from 2 to 5 cells. Somas were round, oval or triangular in shape. The following cortical features were investigated: area, perimeter, and roundness or eccentricity expressed as the ratio of greatest width to greatest length. Preliminary observations, mean ± standard deviation, examined neurons in each of 3 planes: horizontal (N=55), sagittal (N=50), and transverse (N=50). Measurements were as follows: (1.) area (μm²) = 196.88 ± 46.13 – horizontal; 205.58 ± 50.69 – sagittal; and 202.93 ± 39.12 – transverse; (2.) perimeter (μm) = 55.32 ± 7.64 – horizontal; 56.46 ± 7.35 – sagittal; and 54.30 ± 5.70 – transverse; (3.) roundness; 0.675.13 – horizontal; 0.62 ± 0.10 – sagittal; and 0.71 ± 0.11 – transverse. Additional observations will be made to determine whether these morphological features identify subpopulations of relay cells in nucleus rotundus.

A COMPARISON OF THE EIMER'S ORGANS OF THE STAR-NOSED MOLE (CONDYLURA CRISTATA), THE HAIRY TAILED MOLE, (PARASCALOPS BREWERI), AND THE EASTERN MOLE (SCALOPUS AUSTRALIS). K. C. Catania* Neurobiology Unit, Scrips Institution of Oceanography and Dept. of Neurosciences, School of Medicine, Univ. of Calif., San Diego, La Jolla, CA 92030-2011.

Eimer's organ is a tactile sensory structure found on the snouts of moles and some shrews. It consists of a raised dome of epithelium containing a column of cells associated with sensory receptors. Scanning electron microscopy and light microscopy were used to compare the size and distribution of these organs in three North American moles.

Eimer's organs are visible on the skin surface of the hairy tailed mole and the eastern mole. The organ has an ovoid appearance, distribution, and internal structure of the Eimer's organs of the hairy tailed mole are similar to those most commonly found in other species. The structure of the Eimer's organs of the star-nosed mole and the eastern mole diverge from this basic form in seemingly opposite directions. The Eimer's organs of the star-nosed mole are more numerous, smaller, highly organized units with little variability and a consistent pattern of terminal swellings within a cell column, just below a thin keratinized epidermis. By contrast, the Eimer's organs of the eastern mole lie below a thick keratinized epithelium, are larger and more variable in structure, and have no central cell column. These differences may be influenced by the soil type through which each species burrows; saturated mud allowing a more elaborate and delicate sensory apparatus in the case of the star-nosed mole, and dry abrasive soil requiring a thick keratinized epidermis to resist the receptor complex in the case of the eastern mole. Supported in part by NIH grants NS24869 to R. Glenn Northcutt, and 5132-GM08107 (Biological Training Grant).
579.3


The organization and fiber connections of the dorsal column nuclei (DCN) of the anurans Rana ridibunda and Xenopus laevis are studied with immunohistochemistry and tract tracing. Primary afferents from cervical, thoracic and lumbar spinal dorsal roots reach the DCN somatotopically arranged. Primary afferents from the cranial nerves V, VII, IX and X innervate the DCN via the descending trigeminal tract. Nonprimary afferents arise bilaterally in cells of the rhombencephalic reticular formation, the mesencephalic reticular formation, the octavolateralis and thalamic nuclei, and project to the spinal cord.

The main efferent projection of the DCN, i.e. the medial lemniscus, is formed by DCN fibers that cross the midline at the level of the nuclei and innervate contralaterally the rhombencephalic reticular formation, the octavolateralis, cerebellum, the principal, magnocellular and mainly the laminar nucleus of the torus semicircularis, the anterodorsal and anteroventral (including the red nucleus) mesencephalic reticular nuclei and areas and in X, laevis the intermediate and deep layers of the tectum. At mesodiencephalic levels the medial lemniscal fibers innervate the posterior tubercle, the ventral thalamus (ventromedial and ventrolateral nuclei), and sparsely the posterior and central nuclei of the doral nucleus. Spinal, extratrigeminal, DCN efferents reach the granular layer of the cerebellum and descending effenter fibers innervate the spinal cord. Histochimical and immunohistochemical observations of the DCN area revealed the presence of GABAergic, glycineergic, as well as parvalbumine (in X, laevis also calbindin), and NADPH diaphorase positive neurons in the DCN. Terminal structures positive for 5-HT, CRMP, SP, NPY and NADPH are present in the DCN.

579.5


The nervus terminalis (NT) was discovered more than a century ago in elasmobranchs. Presumed homologs of this cranial nerve have been investigated in many vertebrate classes. However, only a few data are available on the connectivity of the NT in elasmobranchs. We investigated the projections of the NT by means of biotinylated dextran amine (BDA) injections into the olfactory bulb (OB) and rostral telencephalic areas and corresponding nerve tracts to the OB in the thornback guitarfish, Platyrrhinoidis trierisata.

Injections of BDA into the OB revealed labeled fibers entering the forebrain in the NT, proceeding caudally in a mediopetal position adjacent to the rocessus neuroporicus and penetrating septal and preoptic areas. Labelled cell bodies were not observed only in the lateral part of nuclear mass, which is similar to previous studies in other elasmobranchs, above the optic tectum. Injections into the area where the NT enters the forebrain revealed labeled cells in all of the ganglia along the course of the NT towards the lateral part of the OB. Previous work has shown that the NT carries tonic efferent activity [Bullock and Northcutt Neurosci Lett 44(1984)155-160]. The labelled cells we found in the nucleus preopticus are possibly the source of these efferents.

Supported by a DFG grant to MIH and NIH grants to RGN and THB.

579.7


The vestibulolateral cerebellum of the catfish is comprised of two discrete granule cell nuclei; a lateral (ELG) and medial (ECM) eminencia granularia. Previous studies have demonstrated that these two areas receive different nerve fiber input from lateral (mechanosensory and electroreceptive) and auricular nerve respectively. Azores of granule cells in the eminencia form a portion of the cerebellar crest that projects back over the medullary octavolateralis nucleus and modulates the activity of ascending medullary sensory neurons. The purpose of this study is to determine some of the sources of afferent input to the eminencia granularia.

Chromed catfish were anesthetized with MS-222 and a division of the eminencia granularia were labeled with either horseradish peroxidase or biot. After suitable transport times the brains were sectioned and viewed via light or fluorescence microscopy.

The bilateral eminencia granularia are heavily interconnected by commissural fibers that decussate through a deep cerebellar commissural dorsal to the cerebral aqueduct. Additional afferent arises bilaterally from the inferior olivary nuclei from two basal medullary nuclei. In the catfish the nucleus is located in the inferior olive, the other is positioned more rostrally and laterally. In the metacetohelicus both the nucleus praeminentialis and the nucleus of the medial longitudinal fasciculus provide labeled terminals to the EC.

The eminencia granularia is part of a descending central control system that provides gain control and adaptive filtering to the octavolateralis sensory systems. Our results indicate several sources of afferent input to this cerebellar region but no role in evoking networks of the SG.

The eminencia granularia is a part of the sensory system.
500.1

COMPARATIVE LOCALIZATION OF NITRIC OXIDE SYNTHASE IN THE VERTEBRATE SPINAL CORD. G. Brüning,*
Department of Anatomy, Free University of Berlin, D-14195 Berlin, Germany.

The localization of nitric oxide synthase was investigated in the spinal cord of mouse, chicken, turtle, frog and goldfish by NADPH diaphorase histochemistry. Specificity of the histochemical detection method was verified by co-localization with an antiserum against nitric oxide synthase purified from porcine cerebrum. In all vertebrate species, the motoneurons were, as a rule, unstained. Strongly stained neurons were concentrated in the dorsal horn. Widespread areas of the dorsal horn were filled by a dense terminal plexus which appeared to be formed chiefly by intrinsic neurons as dorsal root fibers were mostly negative. Internally stained neurons were also scattered in the intermediate grey and the ventral horn. In the mouse, intensely positive neurons were clustered in the preganglionic sympathetic and parasympathetic nuclei. In the corresponding cell columns of nonmammalian species, stained neurons were detected only occasionally. In summary, these results indicate that, despite certain species differences, there is a considerable conservation in the expression of nitric oxide synthase in the spinal cord, suggesting that nitric oxide may influence sensory information processing throughout vertebrate species.

500.2

DISTIBUTION OF THE SUPEROXIDE DISMUTASE 1 (SOD1) IN THE MAMMALIAN NERVOUS SYSTEM. C. Pandey*, Z.-S. Xu*, D.W. Cleveland*, and D. Price*, Departments of Pathology, Biological Chemistry, Neurology, and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

SOD1, an enzyme participating in the free radical scavenger system, protects against oxidative stress. Recent evidence indicates that some cases of familial amyotrophic lateral sclerosis are linked to mutations in SOD1, but it is not clear how these mutations cause degeneration of motor neurons. A first step in understanding these processes is to define the cellular distributions of SOD1 in the nervous system. To this end, we have produced new specific antibodies to be used in Western blot and immunocytochemical studies. In human, monkey, baboon, rat, and mouse, SOD1 immunoreactivity (SOD1-IR) was identified primarily in neurons in the CNS and PNS. In spinal cord, SOD1-IR was present in motor neurons, interneurons, and diffusely in the substantia gelatinosa. Immunoelectron microscopic analysis disclosed that SOD1 was localized predominantly in the perikaryon, axonal and dendritic compartments, and euvacuomous areas of the nucleus, and there was evidence of different levels of SOD1-IR among cell groups. In the hippocampal formation, neurons of the CA3-CA4 sectors were intensively SOD1 immunoreactive, whereas CA1 neurons showed less immunoreactivity. SOD1-IR was also demonstrated in large- and medium-sized pyramidal neurons of roccortex, subsets of neurons in pyriform cortex, amygdala, striatum, and thalamus. These findings indicate that SOD1 is present in many neuronal populations throughout the nervous system and that certain subsets of nerve cells, including motor neurons in spinal cord and brainstem, are enriched in this enzyme.

500.3

NITRIC OXIDE SYNTHASE IN THE LOCUS COERULEUS OF THE CHICK: DEVELOPMENT, DISTRIBUTION AND LACK OF CORRELATION WITH TRANSMITTER PHENOTYPES. A. Scholer*, C. Burgard*, and C.S. von Bartheld*, Departments of Physiology & Biophysics (SJ-40), University of Washington, Seattle, WA 98195; Department of Anatomy & Cell Biology, University of Heidelberg, D-69120 Heidelberg, FRG.

The distribution and morphology of nitric oxide synthase (NOS)-containing neurons was investigated in the locus coeruleus complex (LC) of chick embryos. The NADPH-diaphorase technique was used to detect the presence of NOS at the incubation days E8, E12, E14 and 14 days posthatch (P14). The first NOS expression in LC was found at age E12. The number of NOS-positive cells in the locus coeruleus proper (LCP) increased from E12 to E18 (450 to 850) and decreased slightly after hatching (P14). In the locus subcoeruleus (LSC), NOS-containing neurons could be identified as early as E18. The number of NOS-expressing neurons increased from E18 to P14 (70 to 270). To determine which cholinergic neurotransmitter phenotype may be coexpressed with NOS, adjacent sections were immunolabeled for choline acetyltransferase (ChAT), for somatostatin (SOM) and for the low-affinity neurotrophin receptor (P75) as a marker for noradrenergic LC neurons (von Bartheld and Burgard, 1992; J.Comp.Neural. 320:479-500). In addition, NADPH-diaphorase histochemistry was combined with immunocytochemistry of P75, ChAT and SOM in the same sections. NOS is not co-localized with the P75 receptor (noradrenergic cells) or ChAT but with few cholinergic cells (10%) were double-labeled with NOS. In birds, NOS is an excellent marker for a major subpopulation of LC neurons. None of the classical neurotransmitters examined is coexpressed in NOS-positive LC neurons. These results indicate that major differences exist between species, as the rat LC contains only few NOS-positive cells (Xu et al., 1994, Exp. Brain Res. 95:154-158). The role of nitric oxide in developmental and physiological aspects of the LC in the chick brain. Supported by DHG grant Scho 489/1-1 and NIH grants NS 30305 and HD 29177.

500.4

DISTRIBUTION OF CATCHEMELIN IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF THE LIZARD ANolis SAGrei. A. Rehn, P. Ekström and T. Ousthilm*, Dep. of Zoology, Univ. of Lund, Sweden. Adults of the “brown anole”, Anolis sagrei (Lam., Iguanidae, order Squamata) caught in Miami, Florida, USA and juveniles obtained from our own breeding colony were used in this study. Immunohistochemical methods with antibodies against the transmitter dopamine (DA) and the synthetic enzymes, tyrosine hydroxylase (TH), dopamine β-hydroxylase (DBH) and phensylethanolamine-N-methyltransferase (PNMT) were used to locate the distribution of cathecholamine in the anole CNS. Cathecholaminergic neurons were observed in all parts of the brain. In the olfactory bulb, TH and DA immunoreactive (ir) neurons were observed in the glomerular layer and the external plexiform layer. In the telencephalon, THir neurons were seen in the nucleus septalis medialis. In the diencephalon, THir and DAir neurons were present in the periventricular preptic area, the periventricular hypothalamic nucleus and the lateral hypothalamic area. CSF-contacting neurons in the periventricular hypothalamic organ were only DAir. THir neurons were present in the posterior diencephalon. Large groups of cathecholaminergic neurons were found in the mesencephalon. THir and DAir neurons were located in the ventral tegmental area, the substantia nigra and the pretectal regions. ChAT and SOM were seen in different areas of the cerebral aqueduct. In the rhombencephalon, THir, DAir and DBHir neurons were found in the hindbrain, the nucleus of the solitary tract and in the area postrema. THir, DAir and DBHir neurons were observed in the lateral part of the reticular formation. In the spinal cord, THir and DAir CSF-contacting neurons were present in the ventral wall of the central canal. The distribution of cathecholaminergic neurons in A. sagrei is generally similar to that in previously studied reptiles (Anolis carolinensis, Gekko gecko, Varanus exanthematicus, Chameleon Cup, Pseudemys scripta elegans and Python regius). However, a number of interspecific differences were observed. Supported by grants from the Swedish Natural Science Research Council.
COMPARATIVE RESULTS


The aim of the present study was to extend our knowledge of catecholamine systems (CA) in amphibians to the gymnophonans, i.e. the gymnophonans. For that purpose, the distribution of CA neuronal structures in the permanent aquatic gymnophonan Tiphlocyclus compressuscaudatus was studied by immunochemistry for tyrosine hydroxylase (TH). The TH-immunoreactive cells of this species are located in the olfactory bulb, preoptic area, hypothalamus, pretectum, mesencephalic tegmental areas, and the diencephalon and mesencephalon. A large number of TH-immunoreactive (TH) cell groups were found. They are located in the olfactory bulb, preoptic area, hypothalamus, pretectum, mesencephalic tegmental areas, and the diencephalon and mesencephalon. Nevertheless, when the distribution patterns of TH immunoreactivity of representatives of the three amphibian orders (Anura, Urodela and Gymnophiona) are compared with each other, it is obvious that there exists a basic pattern. Moreover, the organization of the CA in Tiphlocyclus resembles the pattern of TH nerve elements in these species, which may indicate evolutionarily conserved patterns of neuronal organization.

(Supported by DGICYT PB90-0628 and NATO CRG 910907)


The brains of the lizard Anolis carolinensis were either frozen in tissue freezing medium or dehydrated, cleared, and embedded in wax. Following sectioning, 5 - 12 µm, brains were incubated at 4°C for 48 h with a polyclonal rabbit-anti-human glial fibrillary acidic protein antibody (GFA-P). This antibody was raised against amino acids 341 - 367 of the amino terminal domain of human glial fibrillary acidic protein. Receptor staining was accomplished by diaminobenzidine reaction to a biotinylated secondary antibody (goat-anti-rabbit). Most immunoreactive staining was localized in cell nuclei.

The area with the greatest concentration of glial fibrillary acidic protein immunoreactive staining was the anterior dorsal ventricular ridge. This area has also been referred to as the hypopallium and hyperstriatum ventrale. The area of the pallium was seen to be the ventral margins of the lateral ventricles at the level of the optic chiasm and epiphysis. More anteriorly, dense staining was seen in the marginal layer of the dorsal ventricular ridge between the lateral and olfactory ventricles. Staining was also prevalent in the medial cortex, also referred to as the hippocampal cortex or hippocampus par dorsalis.


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SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994

Neuropeptide Y (NPY), a 36-amino acid peptide, has been shown to be localized in neurons of the cerebral cortex, striatum, and amygdala of mammals. The present study examined its distribution in the forebrain of the painted turtle (Chrysemys picta). We used fluorescence immunohistochemistry to visualize the NPY cells and fibers. Sections were incubated in a polyclonal primary antibody against synthetic NPY made in rabbit (Accurate) and then in a biotinylated anti-rabbit secondary, followed by avidin conjugated to the fluorophore FITC. In the telencephalon, NPY-positive cells were found in the tuberculum olfactorium, in the medial, dorsomedial, dorsal, and lateral cortices, and in the dorsal ventricular ridge. NPY-positive cells were also found in the paleostriatum augmentum (equivalent to the striatum), in area (the septal equivalent of nucleus accumbens), and in the amygdala. Few cells were seen in globus pallidus or in the septal area. NPY-positive fibers were widely distributed throughout the telencephalon, but were especially prominent in the molecular layer of the medial and dorsomedial cortices. In the diencephalon, NPY-positive cells were seen only in the dorsal nucleus of the ventral thalamus, which is equivalent to the thalamic reticular nucleus of mammals. Fibers were present in the hypothalamus, including a dense plexus in the nucleus ventromedialis hypothalami. These results are similar to those seen in mammals, especially in the telencephalon, and together with results of studies of other nonmammals, show continuity in forebrain development during the course of evolution. Supported by NS17883 to ASP.

BRAIN METABOLISM AND BLOOD FLOW: BASIC MECHANISMS II


rCBF was measured twice in six dogs within one week. The first measurement was done while animals were sedated using Pentothal, a short acting barbiturate, 20 mg/kg and in the second Fentanyl was titrated to a maximum of 50 doses. The rCBF was measured using a modified NOVO 10a Cerebograph using two detectors on each side; the remaining detectors were shielded. Animals were sedated, endotracheally intubated & connected to the cerebograph. ECG, heart rate, respiration and tidal volume were monitored. The end tidal CO2 was kept at 35-40 mmHg. Radiolabeled Xenon (Dupon Pharmaceuticals) was mixed with low dissolved oxygen saline (LDO). Following registration of background activity for 0.5 min, a bolus of Xe was in an intracranial vein. Xe clearance was recorded throughout 11 minutes and expired air was monitored to supply the air curve. CBF results were expressed in ml/100g/min as the initial slope index between 30 and 90 sec, as CBF15 and as the fast compartment flow FI. Corrections for hemoglobin and CO2 were not instituted. Hemispheric CBF was treated as the average value of the two detectors on each side & an average for each animal was calculated. ANOVA showed a difference in rCBF between the two measurements as described in ISI, CBF15, and FI. There were no differences in PCO2, hemoglobin levels or the injected Xe


Sympathoexcitatory reticulospinal neurons of RVL are central oxygen detectors rapidly inhibited by ischemia, hypoxia, or NaCN (Sun and Reis, J. Physiol. 476:101, 1994). As a consequence, they increase sympathetic nerve activity and arterial pressure (AP) (Sun and Reis, Am. J. Physiol. 268:R245, 1994). The facts that systemic hypoxia increases rCBF without metabolism, stimulation of RVL (Underwood et al., J. Careb. Blood Flow Metab. 12:844, 1992) replicates this response, and bilateral lesions of RVL reduce, by over 50%, hypoxic vasodilation (Underwood et al., Brain Res. 635:217, 1994) suggests that hypoxic stimulation of RVL neurons may increase rCBF. To test the hypothesis, NaCN was microinjected (150 pm, 5 nl) into RVL of anesthetized rats. CN rapidly (<2 sec), site-specifically, dose-dependently and reversibly increased rCBF (measured by laser-Doppler flowmetry) to 21±6% (+0.01, n=8) and AP to 138±13 mmHg (p<0.05). After spinalization, NaCN only increased rCBF. CN also increased the numbers of cortical rCBF wave complexes (Golanov and Reis, J. Physiol. 268:R245, 1994) and excited neurons in lamina V which discharge during the negative potential of spontaneous activity (Golanov et al., Brain Res. 635:217, 1994). These results suggest that the effects of hypoxic stimulation of RVL neurons on rCBF and the activity of "vasodilator" neurons of the cerebral cortex are real. The results also support the hypothesis that hypoxic cranial vasodilation is, in part, neurogenic resulting from reflexogenic activation of a pool of cortical neurons by oxygen-sensitive neurons in RVL.


The present study examines the possible correlation between cerebrovascular vasodilator agents and generation of cGMP in guinea pig cerebral vessels. Aciotocin (ACTH), substance P (SP), nitroglucceryl (GT) and sodium nitroprusside (SNP) not only decreased concentration-dependent relaxation of basal artery segments, but also significantly increased the generation of cGMP of cerebral vessels. Neuropeptide Y (NPY) increased the generation of cGMP by 7%-45% of control levels (at 10-7-10-4M of NPY; p<0.05). In addition, NPY (10-4M) induced a transient relaxation of the precontracted guinea pig basilar arteries with endothelium. This transient relaxation was blocked by yohimbine (NOLAG 10-4M). α-Tritostol did not alter the production of cGMP. In the presence of α-tritostol NPY (10-7-10-4M) did not significantly elevate the production of cGMP. The rise in cGMP induced by AC, SP and NTG was slightly increased by the addition of NPY (3x10-5M). AC, SP and NTG induced an endothelium-dependent relaxation of the precontracted guinea pig basilar arteries, while SNP and NTG induced an endothelium-independent relaxation. AC, SP and NTG induced concentration-dependent relaxations of basilar artery, respectively. This relaxation elicited by AC or SP, but not NTG, was markedly attenuated by NPY (3x10-5M). This inhibition effect of NPY on vasoconstrictor responses was completely reversed by α-tritostol (10-4M). In conclusion, there is a close correlation between relaxation and cGMP formation induced by AC, SP, NPY, SNP and NTG. The relaxant response to AC, SP and NPY are mediated via the release of endothelium-derived relaxing factor/taurine oxide (EDRF/NO), while SNP and NTG act directly on the guanylyl cyclase. The inhibitory effect of NPY on endothelium mediated relaxation by AC and SP are not mediated via cGMP, but inhibited by α-tritostol.

RESPONSES OF CEREBRAL COLLATERAL VESSELS TO INHIBITION OF ATP-SENSITIVE POTASSIUM CHANNELS. C.A. Mazzocchi, J. Gerdes, M.G. Muhlen, S.C. Robertson* and C.M. Loftus. Div. of Neurosurgery, Unv. of Iowa College of Medicine, Iowa City, IA 52242 and Veterans Affairs Medical Center, Iowa City, IA 52246.

The mechanism of collateral vasooclusion following cerebral arterial occlusion is not completely understood. The canine cerebral vasculature was investigated for the presence of ATP-sensitive potassium channels, and effects of an inhibitor to these channels as they relate to collateral blood flow. In eight mongrel dogs a left sided craniotomy was performed with temperature controlled artificial cerebrospinal fluid (aCSF) superfused over the brain. Normal cerebral artery pressure was measured with a glass microprobe. A branch of the middle cerebral artery (MCA) was cannulated and back pressure measured. Collateral-dependent branch was identified using the "shadow flow" technique. Glibenclamide (10-5M) was added to the aCSF. Regional cerebral blood flow (rCBF) in subcortical cerebrum was measured with radioactive microspheres. Baseline blood flow to ipsilateral normal cerebrum was 98±16 ml/100g min-1 and did not change during glibenclamide. Flow to collateral-dependent tissue was 93±13 ml/100g min-1, and 68±7 ml/100g min-1 following glibenclamide (p<0.05). The attenuation of collateral blood flow was associated with a profound decrease in cannulated MCA branch back pressure. Pressure in the normal MCA branch was unchanged.

When ATP-sensitive potassium channels, which probably participate in compensatory vasooclusion were inhibited, a reduction in rCBF occurred in collateral-dependent tissue, but not in normal cerebrum. This would imply that ATP-sensitive potassium channels are involved in collateral vasodilatation following MCA occlusion, but may not be involved in maintenance of resting basal tone of cerebral arteries.
581.5

STIMULUS PARAMETERS INFLUENCE MAGNITUDE AND TIME COURSE OF OPTICAL INTRINSIC RESPONSES IN RODENT BARREL CORTEX. A.J. Blood, T.M. Narayanan, A.W. Toga. Laboratory of Neuro Imaging, UCLA School of Medicine, Los Angeles, CA 90024

Optical imaging of intrinsic signals was performed in primary somatosensory cortex of the rat during single whisker deflections of varying frequencies and durations, as well as during competitive stimulation of forelimb and whisker. A dose-response relationship was observed between whisker stimulation parameters and the intensity and spatial/temporal characteristics of the optically recorded intrinsic signal. When frequency was constant, longer durations of stimulation resulted in larger magnitude responses, as determined by pixel intensity in a statistically defined region of interest. Similarly, when duration was constant, higher frequencies of stimulation resulted in larger magnitude responses. In addition, greater numbers of deflections per trial resulted in higher magnitude responses than relatively smaller numbers of stimuli. Conversely, there were no differences in response magnitude between different combinations of frequency and duration which had in common the same number of stimuli. Temporally, there is evidence that the time between stimulus onset and peak response is influenced by frequency, with higher frequency stimulation resulting in a faster time-to-peak. Registration with certain aspects of vascular anatomy and stereotactic coordinates demonstrated that intrinsic responses were well spatially localized with both types of fiducial points. Electrophysiological recordings demonstrated that optical response regions were coincident with areas of neuronal activity, although the time course of optical responses was slower than that of neuronal firing. Finally, competitive forelimb stimulation caused decreases in whisker response magnitude, and the intensity of forelimb stimulation determined the degree to which the whisker response decreased. Thus, both whisker stimulus parameters and introduction of competitive stimuli are critical variables in determining the magnitude and/or timing of optical intrinsic responses in rat barrel cortex.

581.6

FUNCTIONAL NEURAL-VASCULAR CONGRUENCE OVER SOMATOSENSORY CORTEX. S. M. Narayanan*, A.J. Blood and A.W. Toga. Laboratory of Neuro Imaging, Dept of Neurology, UCLA, Los Angeles CA.

We examined the relationship between cerebral blood volume (CBV) and activation of primary somatosensory cortex (S-I) in the rat, using plasma-fluorescence, optical intrinsic signals and single unit recordings. Cortices of urethane-anaesthetised male Sprague-Dawley rats were exposed, and fluorescent Texas Red Dextran dye (MW 70,000) given i.v. Visual deflection or forelimb electroshock were associated with dye-related fluorescence increases over contralateral postero-medial barrel subfield or forelimb S-I. Fluorescence change foci overlay areas of increased cortical layer III cell firing on single unit recordings. Surface boundaries of even the smallest foci overspill the electrolytologic center receptive field. Larger stimuli caused fluorescence foci to enlarge considerably while the receptive field did so only slightly, increasing this spatial discrepancy. Fluorescence change was delayed and prolonged, suggesting that CBV increases at 1-1.5 s, and peaks 2.2-5 s after the onset of 1-2 s stimulation in both regions. Interleaved optical intrinsic studies revealed reflectance decreases between 550-830 nm, with similar timing and location to fluorescence increases after identical stimuli.

Thus, we observed delayed increases in vascular dye-fluorescence (related to CBV) over activated cortex. The striking similarity between optical reflectance and fluorescence suggest that changes in intrinsic cortical reflectance are strongly related to changes in CBV.

581.7

BLOOD FLOW RESPONSES IN THE RAT BARREL CORTEX DURING WHISKER STIMULATION. D. Liu, J. Dowling, M.E. Spence, C. Rovainen, and T. Woolsey*. Dept. of Neuro. and Neurosurg. Surg. of Cell Biol, Dept. of Neuro. and Neurosurg. and Univ. of Medicine, St. Louis, MO 63110.

Arteriovenous transit times (AVTTs) of 0.001% FITC-dextrans were measured by videomicroscopy through a closed cranial window to examine blood flow responses during whisker row and single whisker stimulation. During non-sensory activity during whisking, AVTTs were determined with optical imaging of "intrinsic" responses. Whiskers were stimulated at 4-5 Hz for 1 min. Whisker row stimulation produced a mean AVTT decrease of 24.0% (n=24, SD±11.61, p<0.0001), whereas single whisker stimulation resulted in a mean decrease of 16.5% (n=68, SD±9.0, p<0.0001). Single whisker stimulation produced a significantly smaller decrease (p<0.001) in vessels with different constrictions. Decreases in AVTTs could be detected within 5 seconds of the onset of whisker stimulation. Recovery to baseline values occurred with 5-10 seconds after stimulation ceased. These studies show that targeted measurement of AVTTs can be used for measurements of quantitative localized changes in blood flow in response to natural stimuli.

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581.8


Local cerebral blood flow (LCBF) and local cerebral glucose utilization (LCGU) in response to natural stimulation of the trigeminal (whisker) pathway were determined and its connectivity was studied with optical imaging and fluorescence microscopy. The LCBF and LCGU in the trigeminal (V) pathway were measured autoradiographically with [14C]iodoantipyrine and by 2-deoxyglucose respectively. Stimulation of the V nerve was elicited by: V nerve and tract; V motor root and nucleus; V spinal nuclei and principal nucleus; ventralbasal thalamus and barrel cortex. LCBF and LCGU were measured simultaneously using two methods: LCBF and LCGU in the left barrel cortex. LCGU values of various structures during whisker stimulation were highly correlated (r=0.95) with their corresponding LCBF values. The stimulated regions were indicated by close coupling between LCBF and LCGU during whisker stimulation.

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581.9


Blood flow increases during stimulation of single whiskers in rodent barrel cortex. The control and precision of the vascular response depends in part on the structure of cerebral vascular units: a terminal field found in efferent and venular draining those capillaries. Vascular units were demonstrated in barrel cortex by dye injection into single arterioles. Adult mice were anaesthetised and perfused with 4% paraformaldehyde in HEPES-saline from the abdominal aorta. The aim of fixation was to prevent further constriction or relaxation or reflow of parenchymal elements. Blood vessels were visualized by steady perfusion at 60cm H2O with 1% Propidol Red in 150mM NaCl. Single generating 7-23 mm arterioles were impaled with glass micropipettes and injected with 0.4% Patent Blue or 2% FITC-albumin. Injected markers emerged in 1-7 mm vessels. Vascular units were defined as the polygon of the injected arteriole and its emerging vessels at the pial surface. Arteriolar domains were approximately the same size as projected layer IV barrels stained for cytochrome oxidase but formed independent maps. Portions of arteriolar domains overlapped, and the distribution of internal capillary 10-500um diameters. The injections of internal arteriolar branches may clarify the structure of finer capillary modules in somatosensory cortex.

Supported by NIH Grant NS 27811 and HL 41075, the McDonnell Center for Studies of Higher Brain Function and an award from the Spastic Paralysis Foundation of the Illinois-Eastern Iowa District of the Kiwanis International.

581.10


Intrinsic cortical responses were examined over the rat barrel cortex during continuous whisker stimulation. Videomicroscopy through a closed cranial window was recorded before and during whisker stimulation of C-row and single whiskers. After 4-5 Hz for 10-50 min. Computer analysis of these recordings and of "live" images revealed "intrinsic" responses localized to the appropriate areas of barrel cortex by local histology. Responses remained localized to these areas up to 10 min, but subsequently appeared to spread. The quality of images constructed from video recordings was comparable, though slightly inferior, to live images. These studies demonstrate that "intrinsic" optical signals persist and remain localized for at least several minutes of continuous whisker stimulation. Video tape recording facilities subsequent analysis of "intrinsic" optical responses.

This work supported by NIH Grants NS 17765, NS 27811, and HL 41074, the McDonnell Center for Studies of Higher Brain Function and an award from the Spastic Paralysis Foundation of the Illinois-Eastern Iowa District of the Kiwanis International.
RED CELL FLUXES CHANGE WITH STIMULATION OF RAT WHISKER BARREL CORTEX. E.P. Liang, B. Gillespie, M.P. Spence, D. Lin, C.M. Reynolds, T.A. Woolver and R.E. Schmidt*. Dept. of Neurosurgery, of Cell Biology and Physiology and of Pathology, Washington University School of Medicine, St. Louis MO 63110.

Fluorescent rhodopsins (IRBCs) were used to measure blood flow changes in cerebral cortex with whisker stimulation. IRBCs (PKH2-GL, fluorescein isothiocyanate, and fluorescein beads) are long lived intravascular markers (t1/2 = 2 hours) permitting changes to be evaluated prior to, during and after controlled whisker stimulation. IRBCs were recorded microscopy through a cranial window over rat whisker-barrel cortex and were counted as they emerged from cortical vessels. "Optical intrinsic" signals were used to target activated barrel cortex; after the experiments vessels and barrel vessels were mapped by histology. Stimulation of a row of whiskers promptly and significantly increased flux draining a periactively active cortex; flux recovered quickly to baseline after stimulus termination. Vessels draining non-actively active barrel cortex have reduced or unchanged fluxes. Vessel diameter did not change. Localized changes in RBC flux were consistent with previous measurements by other methods. In contrast to plasma markers, IRBCs permitted continuous evaluations over long periods and allowed selection and detailed sampling of multiple vessels from images = 1mm2.

Supported by a Howard Hughes Fellowship (O.E.L.), NIH Grants NS 28781 and HL 41075, the McDonald Center for Studies of Higher Brain Function and an award from the Spastic Paralysis Foundation of the Illinois-Eastern Iowa District of the Kiwanian International.

COGNITION IV

CORNELL UNIVERSITY


The corpus callosum (CC) can be easily visualized and measured on midsagittal magnetic resonance imaging (MRI) sections. Variations in the size of anatomically defined subregions, such as the genu, body, isthmus and splenium, have been associated with verbal fluency, language lateralization, handedness, sex, and age. In this study these subregions of the CC were measured using Witsett's method. Intrarater and inter-rater reliability estimates ranged from 0.87 to 0.94. We investigated the development of these regions in a group of 32 control children between the ages of 5.7 and 12.5 (18 boys and 14 girls) who had received the Test of Nonverbal Intelligence (TONI), mean score = 106). The area of each region was divided by the area of the total intracranial volume to correct for overall differences in brain size. After this correction, the body of the corpus callosum was the only region that was significantly smaller in girls (F1,59 = 4.62, p < 0.02). The body and the isthmus were the only regions to show a significant increase in size with age (Isthmus: r = 0.42, p < 0.02; Body: r = 0.39, p < 0.03). In girls, the size of the genu showed a trend toward correlation with the TONI score (r = 0.48, p < 0.10). In boys, none of the subregion correlations with the TONI were greater than 0.15. We are currently studying the relation between subregion size and asymmetries in association cortex. (Supported in part by Social and Behavioral Sciences Research Grant No 12 FY93-0551 from the March of Dimes Birth Defects Foundation.)

REFERENCES

ABNORMAL CORTICAL ACTIVATION GENERATED BY SIMPLE AUDITORY STIMULATION AND MUSIC IN CHILDHOOD AUTISM. M. Zilbovicius*, B. Garreau, C. Barthelmy, P. Remy, A. Syrnes, G. Leland and Y. Sannier, SMFI, CELA, Oraey and INSERM U 316, CHU Bretonneau, Tours, France.

Abnormal behavioral and electrophysiological responses to auditory stimuli are typical of childhood autism, and may reflect an abnormal cortical processing of auditory inputs. We investigated a putative abnormal pattern of cortical activation in autistic children using an auditory stimulus (CBF paradigm. Eleven autistic children (mean age 8 ± 3.5 years) were compared to five non-autistic children (mean age 8 ± 3.5 years). CBF was measured under premedication using SPECT and 110mXenon (at rest) and 2) during simple binaural auditory stimulus (70 Hz tones, duration of 200 msec, intensity of 80 dB, frequency of one tone per second) and 3) while listening to music (Schubert piano sonata no 9). No CBF abnormality was observed at rest. The simple auditory stimulus induced a rCBF increase in the left temporo-occipital cortex in controls (+5% ± 3%; p<0.005). Listening to music induced in autistic children a rCBF increase in the right temporo-occipital cortex (+3% ± 4%; p<0.005). Autism showed a rCBF increase in the right temporo-occipital cortex (+5% ± 4%; p<0.005). Comparison between both groups revealed a significant difference in the left temporo-occipital region (p<0.005). Listening to music induced in autistic children a rCBF increase in the right anterior temporal (+5% ± 4%; p<0.001) and in the temporo-occipital region (left: +6% ± 8%; p<0.02; right: +6% ± 12%; p<0.05). Children showed a trend toward activation in the left temporo anterior region (+3% ± 5%; p<0.09). Comparison between both groups revealed a difference in the right anterior temporal region (p<0.05). Thus, autistic children seem to have an abnormal lateralization pattern of cortical activation induced by auditory stimulations, with deficient left cortical processing of short simple auditory stimuli, and increased right cortical processing of music. Supported by France-Telecom and INSERM network # 489001.
582.5 VISUAL RECOGNITION MEMORY IN HUMAN INFANTS. O. Frascati* and S. de Schonen. Developmental Unit, Lab. of Cognitive Neuroscience, C.N.R.S., Marseille, France.

Visual recognition memory was assessed in 3-day-, 2-month-, and 3-month-old infants with a paired-comparison task. Infants were first habituated to a picture of a face using an infant-controlled procedure, and, after a 2-min retention interval, they were presented with the familiar face and a novel face for two retention periods of 16 sec for the youngest group and 20 sec for the oldest groups. Time spent looking at either stimulus during the two retention periods was measured and percent fixation to the novel face was calculated. At the age of 3 days and 3 months, infants looked significantly longer at the novel face (p < .01) whereas at the age of 2 months, infants looked longer at the familiar face (p < .01). These data demonstrate the presence of visual recognition memory for delays of 2 min as early as 3 days of age in human infants. In addition, the change in preferential viewing in 2-month-old infants (performance for familiar instead of novel face) suggests a discontinuity in the development of this memory system. Together with the lack of novelty preference found in amnesic patients (Mckee & Squire, 1992, J. Exp. Psychol.) and in infant monkeys with cerebral temporal lobectomies (Bachevalier et al., 1993, Neuroreport), the findings indicate that, in human neonates, the medial temporal lobe makes a critical contribution to recognition memory. These results are discussed in relation to neuroanatomical data on the maturation of the visual system and medial temporal lobe structures in human and non-human primates.

582.7 AUDITORY EVOKE POTENTIALS AND VERBAL AND SPATIAL ABILITIES IN PREADOLESCENT CHILDREN. J.J. Shurett*, D.W. Shurett, G.L. Clark, C.P. Scheffer, R.L. Chepper, T. Quinlivan, M.L. Voorhees. Departments of Neurology and Pediatrics, SUNY at Buffalo School of Medicine, 100 High Street (D6), Buffalo, NY 14203.

Evidence from both behavioral and electrophysiological studies has supported the notion that there are differences between the sexes in both anatomical/functional brain organization as well as in cognitive abilities, such as visual spatial perception and memory. Our laboratory has been exploring these purported cognitive sex differences using both neuropsychological and electrophysiological techniques. In the present study, we examined the pattern of cerebral activations across a well characterized sample of pre-adolescent males and females in order to further explore possible relationships between cognitive abilities and electrophysiological indices of cortical organization.

Boys and girls (N=15) between 8 and 11 years of age were studied. Children were determined to be prepubertal by the Tanner Scale of Puberal Development. Subjects participated in (a) neuropsychological assessment and (b) electrophysiological testing to obtain auditory evoked potentials (AEPs) to probe stimuli from Frontal, Temporal, Central and Parietal scalp sites during the performance of spatial and verbal tasks.

Subjects were categorized into high, medium, and low spatial abilities groups based on a linear combination of three behavioral measures of visual-spatial skills. Differences in AEP amplitude occurred between the high and medium versus low spatial abilities groups obtained during the performance of spatial tasks. High and medium spatial subjects showed greater amplitude AEPs as compared to the low spatial subjects. These differences were most prevalent in the parietal leads and were not seen during the verbal task. High spatial subjects, regardless of sex, showed this AEP pattern. Supported in part by NIH grant HD25718.


Previously, Kimura and Carlson (1993) reported a correlation between cognitive pattern and dermatoglyphic patterns on tasks involving spatial abilities and sex differences in performance. Specifically, both male and female subjects who had a greater number of loops in the right thumbprint compared to the left thumbprint performed better on cognitive tests usually associated with enhanced performance by males (e.g., spatial processing, math). In contrast, subjects with higher left-hand counts did better on tests frequently associated with superior performance by females (e.g., fluency, perceptual speed). In this preliminary attempt to replicate Kimura and Carlson's findings, young adult male and female subjects performed a variety of paper-and-pencil cognitive tasks and their thumbprints were examined. As expected, based on earlier work, the males, overall, had significantly more thumbprints than did the females. We also found that all subjects performed equivalently on a putative gender-related task (i.e., vocabulary). On three tests of spatial ability, however, there was a dissociation of dermatoglyphic and memory patterns. Specifically, the Children's Digits Test showed no relationship between dermatoglyphic asymmetry and performance. The Full-Memory Test showed a greater relationship between dermatoglyphic asymmetry and performance, whereas the Schaffer Task showed a stronger performance for females with right-handedness. The dermatoglyphic asymmetry was not related to a difference in memory performance. The Full-Memory Test showed a greater relationship between dermatoglyphic asymmetry and female performance, whereas the Schaffer Task showed a stronger performance for females with right-handedness. The results suggest that dermatoglyphic asymmetry may play a role in the development of spatial abilities.

582.10 SEX DIFFERENCES, SPATIAL WORKING MEMORY AND EVENT-RELATED POTENTIALS. J.M. Hoering*, S. Levine, R. You, & C. Edgar. Dept. of Psychol., Univ. of New Mexico, Albuquerque, NM 87131 and Radiology, V.A. Medical Center, Albuquerque, NM 87123.

Research on individual differences in cognitive functioning is beginning to reveal important sex differences in problem-solving strategies. It has been found that males outperform females on certain tasks that require imaginary manipulation of objects, navigating through a route, guiding or intercepting projectiles, and mathematical reasoning tasks. Females do better on tasks in which perceptual speed is important (e.g., identifying matching items). They have greater verbal fluency, are superior in arithmetic calculation, recalling landmarks along a route, and manual tasks that require fine-motor coordination. Here we report a novel sex difference. The task, based on a procedure described by Sternberg (1969), is presented in two phases. Phase 1 requires the subject to memorize an array of letters. The locations occupied by letters in an array in phase 2 is then the subject is shown single letters and is asked to identify whether the letter a) was a member of the memorized array (letter identification), or b) a letter occupied by an letter in the memorized array (location identification). Females take the same amount of time for letter identification as for location identification of a letter that is faster in the location versus letter identification conditions. This suggests that males use different processing mechanisms to solve location versus item-recognition memory tasks. Females appear to use a common processing mechanism for the two tasks.
582.11  
GENDER DIFFERENCES IN SENSORY INTERHEMISPHERIC TRANSMISSION TIMES. S. Caillé and M. Lavandière*. Groupe de Recherche en Neuropsychologie Expérimentale, Univ. de Montréal, Qué., Canada.

The fibers of the corpus callosum (CC) connect the hemispheres following a rostro-caudal distribution: visual fibers course through the splenium, auditory fibers through the posterior body (isthmus) and somesthetic fibers through the anterior body. The aim of this study was to estimate the interhemispheric transmission time (TTT) in the visual, auditory and somesthetic modalities in right-handed women, given that previous data suggest a gender dimorphism favoring women in the posterior part of the CC (spleminum and isthmus). Sensory ITTs consisting of a tone at threshold, puffs of pure tones were presented to 30 normal dextral subjects (25 males and 25 females) to evaluate, visual, somesthetic and auditory ITT respectively. Results indicated that while somesthetic (13.61 ms) and visual (3.75 ms) ITTs did not differ according to gender, auditory ITTs of men (2.52 ms) and women (0.09 ms) differed significantly. This finding is cautiously interpreted as the consequence of women having a larger isthmus than men. Moreover, it offers an anatomical substrate for the reported weaker lateralization of function in women.

582.12  
SEX DIFFERENCES IN NUMERICAL DENSITY OF NEURONS IN HUMAN AUDITORY CORTEX. S. F. Winblad1, I. I. Glezer2 and D. L. Kigar3. 1Dept. of Psychology, McMaster Univ., Hamilton, ON, L8N 3Z5; 2Dept. of Neurology, Univ. of Cincinnati, Cincinnati, OH; 3Dept. of Neurology, Sunnybrook & Women's Hospital, Toronto, ON, Canada.

Brain size is approximately 10% larger in men than women. This sex difference could be reflected in some microscopic difference: e.g., in the total number fibers, number or numerical density of neurons, differences have been documented in the gross anatomy of and in the cognitive functions mediated by paraietotemporal regions (Winblad & Kigar, JCN, 1992). The aim of the present study was to assess microscopic features of cytoarchitectonic area T1 (von Economo), auditory association cortex in the parietal temparate within the Sylvian fossa. The brains of 5 women and 4 men were studied (mean age = 54 and 49 yr, respectively), all selected to be of documented normal neurologic and cognitive status and consistently-right-handed. Cortical depth (D), the number of neurons in a column under 1 mm surface area through the depth of the cortex (N_D), the number of neurons per unit volume (N_v) were obtained using direct counting under the light microscope. Counts were made from 25μm Nissl-stained sections from right and left hemispheres. These three measures were also obtained for each of the 6 cortical layers. Results indicated that regardless of hemisphere, D did not differ between sexes, but N_D did by 11% (df = 7, p < .008, two-tailed t-test). There was no overlap between the sexes. This sex difference was observed for layers II and IV only. These findings suggest that sex differences in architecture and, as a consequence, possibly in neurophysiology, are mostly non- pyramidal, granular layers associated with reception and distribution of information to the cortex. Such a sex difference could have consequences for neural function and cognition.

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582.13  
SEXUAL ORIENTATION AND ANATOMY OF THE CORPUS CALLOSUM. A. Scammongela1, S. F. Winblad1, M. Bronkelli2, P. Stachevska3, S. Black4, G. Ouma5, M. Stonier6 and B. Buck6. 1Dept. of Psychiatry, McMaster Univ., Hamilton, ON, L8N 3Z5; 2Dept. of Medical Imaging, Medicine (Neur.) and Rad., Sunnybrook Health Science Centre, Univ. of Toronto.

An increased prevalence of left handedness (defined as not exclusive right-hand-preference) was found in gay men compared to the general population (McCormick & Winblad, Psychoneuroendocrinology, 1991). Left handedness and, by inference, hemispheric functional asymmetry, was found also to be correlated with increased cross-sectional area of the isthmus of the corpus callosum in men (Winblad & Goldsmith, Brain Res., 1991). Based on these and other findings it was hypothesized that the isthmus is larger in gay than heterosexual men even when hand preference was controlled. Eleven gay men, 10 heterosexual men matched for age and educational level, all selected to be in good health and consistently-right-handed, underwent a research MR scanning procedure specifically designed for this study. Callosal measurements were obtained from 4 mm mid-sagittal slices. Area measures of the total corpus callosum, the isthmus and other subregions were obtained. The isthmus was larger in gay men by 13% (p = .05, one-tailed t-test). Similar results were reported for the anterior commissure (Allen & Gorski, PNAS, 1992). The isthmus interconnects right and left parietotemporal regions involved in linguistic and spatial cognitive functions, tasks on which gay and heterosexual men differ (e.g., McCormick & Winblad, 1991). Our findings add to the evidence of a neurobiological factor in the origin of male sexual orientation. Moreover, our results indicate that sexual orientation is linked to functional lateralization of higher cognitive functions and may be part of a larger constellation of cortical and cognitive characteristics.

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582.14  
SEMANTIC INTERHEMISPHERIC INTEGRATION IN THE SPLIT-BRAIN: MORE ILLUSORY THAN REAL. Alan Kinsbourne and Michael S. Gazzaniga*. Center for Neuroscience, University of California, Davis, 95616.

Subcortical semantic transfer is strongly suggested when a callosotomy patient draws pictures that integrate the meaning of words lateralized to the separated hemispheres. In our study patient J.W. drew pictures with the left hand (right hemisphere) or the right hand (left hemisphere), with or without visual feedback of the drawing. Sometimes the presented words were conceptually ambiguous (e.g., "word" and "stool" may be drawn as a toad sitting on a stool, or as a single emergent object -- a mushroom). Results indicated that word integration (e.g., a toad sitting on a stool) occurred mainly when visual feedback was provided; hemispheric control of the ponslare as well as the contralateral response hand was observed; and emergent objects were never drawn when words were lateralized. We conclude that what might first appear as evidence of subcortical semantic integration is in fact each hemisphere drawing its own separate picture with the same response hand on the same piece of paper. Our results support the view that the evidence for interhemispheric semantic integration in the split-brain patient is not compelling. (Supported by NIH/NINDS P01 NS1778).

582.15  
THE RELATIVE VALUE OF SPEECH ARREST, READING AND REPEITION ERRORS TO DETERMINE CEREBRAL DOMINANCE FOR LANGUAGE DURING THE INTRACAROTID SODIUM AMYTAL TEST. J. Cabot, H. Roquis*, A. Toutard. Université du Québec à Montréal & Hôpital Notre-Dame, Montréal, Québec, Canada.

The intracarotid Sodium Amytal (ISA) test is commonly used to establish cerebral dominance for language (CDL) prior to surgery for intractable epilepsy. CDL determined clinically is based on a general analysis of the operative reactions during the ISA test but the respective lateralizing value of speech arrest, reading and repetition errors has not been fully examined. To address this issue, the ISA sessions of 30 (clinical CDL: 25 left (L), 2 right (R) and 3 mixed) right-handed epileptic patients, with a unilateral temporal focus, were examined retrospectively. For each patient, presence of speech arrest and dysphasic errors during reading and repetition of words and phrases were compared for L and R injections. Speech arrest was observed in 28 patients (L: 21; R: 7); L > R only; 3: L = R; 4: The presence of suppression corresponded for all patients but 2 with (mixed dominance) with the established clinical CDL. The analysis of 114 reading errors produced by the patients with a L CDL revealed the expected dissociation between neglect error (R inj.) and phonemic paraphasia (L inj.). However, morphological errors were found after both injections. In contrast, repetition errors were observed only following the dominant injection and were mostly phonemic transformations. Taken together, these results suggest that speech arrest and repetition errors are specific to the injection of the dominant hemisphere. While reading errors are not, under these findings would apply to all epileptics (e.g., left-handers, frontal focus etc.) will also be discussed.

582.16  

We examined hand size in 50 adult volunteers who described themselves as right-handers, and 50 who considered themselves left-handers. Volumetric measurements of the two hands, assessed for each subject by a simple water displacement technique, showed that right-handed individuals have larger right hands than left (mean difference = 3.5 ± 0.4%, p < 0.000001). In contrast, the hands of left-handers are much more nearly symmetrical (mean difference = 0.3 ± 0.5%, p > 0.5). Based on what is known about trophic interactions between neurons and their targets, these findings predict a corresponding asymmetry of the relevant parts of the sensorimotor system in right handers. This implication accords with preliminary studies of the human sensorimotor cortex, which show a lefthand hemispheric asymmetry in the region where hand and upper limb are represented (White et al. Nature 368: 197-198, 1994). Corresponding studies of the spinal cord show a rightward asymmetry at the level of the cervical enlargement (mean difference of the cross-sectional area of the hemisected = 6.3 ± 2.7%, n = 5; see also Nathan et al. Brain 113: 363-324, 1990). Measurements of hand size and neurosonomic asymmetry in infants could address whether these asymmetries arise from differential use or intrinsic factors.
FUNCTIONAL HEMISPHERIC ASYMMETRY IN HUMANS:
METABOLIC EVIDENCES OF DIFFERENT CONNECTIVITY IN FOCAL HIPPOCAMPAL LESION. P. Parker and M.E. Lévesque.

583.1

RECOVERY OF A VIBRISSEAE-DEPENDENT BEHAVIORAL RESPONSE FOLLOWING PHOTOTHERMニック INFARCTION OF SOMATO-SENSORY CORTEX IN RATS. A.J. Parent, M.D. Magarey, A. Ferri, P.M. McCabe, W.D. Dietrich, E.J. Gage. Dept of Psychology, University of Miami, Coral Gables, FL 33124

Previous work indicates that rats trained to perform an appetitively motivated vibrissal sensory discrimination task exhibit substantial behavioral deficits following an infarction of the primary somatosensory cortex (S1). These deficits were monitored using a water maze task (goal: a six-sided cylinder, one side open) and the one-way discrimination (control: S1, experimental: non-S1). Sixteen adult male Wistar rats were trained to jump into one of the two open corners of the cylinder for a food reward, and were then tested in a water maze task until they met the criterion for escape behavior. After the training phase, rats were divided into two groups: control (n=7) and experimental (n=7). Following the training phase, the experimental group was subjected to a unilateral photothermal infarction of the S1 cortex, while the control group was subjected to a sham procedure. Post-infarction behavioral testing revealed that animals in each of the infarcted groups required a significantly greater number of days to return to criterion than did sham rats. Nevertheless, each animal eventually recovered to pre-infarct levels of responding. These results indicate that, even after a large infarct, some aspect of behavioral recovery is still possible. The mechanism of behavioral recovery requires neural plasticity in that region. Supported by AHA 93GIA60

583.2

NEURAL PLASTICITY I

583.3

MK-801 FAILS TO INDUCE RECOVERY FROM CHRONIC TACTILE PLACING DEFICITS AFTER UNILATERAL LESIONS INCLUDING THE ROSTRAL AND CAUDAL FORELIMB AREAS OF THE RAT SOMATOSENSORY/MOTOR CORTEX. S. Batha* and T.M. Bark, Dept. of Psych., Texas Christian University, Fort Worth, TX 76129

Previous studies have shown that unilateral lesions of the caudal forelimb area (CFL) of the rat somatosensory motor cortex (SMC) produce a transient ipsilateral somatosensory asymmetry on a bilateral-tactile-stimulation test and transient contralateral impairment in tactile-forelimb testing (lasting 14-28 days). Recovery from these deficits is facilitated by a region of the noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 beginning 16 hrs after the lesion. The present study investigated whether a similar regimen of MK-801 would attenuate chronic deficits produced by a unilateral lesion including the rostral and caudal forelimb area (RFL) of the rat SMC. Rats received large unilateral lesions of the SMC followed by a regimen of MK-801 (1 mg/kg) beginning 16 hrs after the brain injury. In untreated control rats, no differences in tactile-forelimb placing deficits were observed, and there was no spontaneous recovery. Control rats also showed an ipsilateral asymmetry on the bilateral-tactile stimulation test that partially recovered in the 6 month postoperative observation period. Treatment with MK-801 failed to induce recovery of tactile-forelimb placing deficits but did significantly accelerate the rate of recovery on the bilateral-tactile stimulation test. These data suggest that while MK-801 may be effective in facilitating the rate of recovery from transient deficits, there is no evidence that this agent has the capacity to enhance recovery when no restoration of function is expected. Moreover, the present results indicate that an intact normal forelimb area may be involved in the process of recovery from unilateral CFL lesions because removing this structure (along with the CFL) prevents the occurrence of recovery.
583.4 EFFECTS OF SCOPOLAMINE AND MK-801 ON RECOVERY OF FUNCTION FOLLOWING SENSORIMOTOR CORTEX LESIONS IN THE RAT. B.M. Sapone*, M.R. Huang, S. Bhatnagar, and L.M. Barth, Department of Psych., Texas Christian University, Ft. Worth, TX 76120.

Following brain injury there is an excessive release of glutamate and acetylcholine which may lead to suppression of brain function and to the development of secondary brain injury. Previous research has suggested that agents which antagonize glutamatergic and cholinergic receptors may serve as neuroprotective agents and facilitate behavior that has been lost. Here, we evaluated the efficacy of treatment with MK-801 (a noncompetitive antagonist at the N-methyl-D-aspartate [NMDA] receptor) and scopolamine (an antimuscarinic agent at the muscarinic acetylcholine receptor) following brain injury, very few studies have systematically examined the "window of opportunity" for these drugs. Moreover, the effects of treatments with a combination of neuroprotective drugs are largely undetermined. The present study examined the "window of opportunity" for MK-801 and scopolamine by themselves and in combination. Rats received unilateral lesions of the frontal cortex representation in the somatosensory motor cortex (SOM) and a single injection of either scopolamine (1 mg/kg), MK-801 (1 mg/kg), scopolamine + MK-801, or saline. In addition, to the type of treatment, the timing of the drug injections was varied among groups with some animals receiving their treatments at 15 minutes, 2 hours, or 48 hours after the brain lesion. Behavioral tests included measures of forelimb tactile placing and locomotor placing. The results were that although scopolamine and MK-801 by themselves facilitated the recovery of function on some of these tasks when given at 15 minutes or 2 hours, the combination treatment provided the most consistent beneficial effects regardless of the type of injection or the behavioral test. These data support the idea that activation of both muscarinic and NMDA receptors are involved in the production of secondary brain damage and that combination treatments with antidepressants may provide the most consistent facilitative effects on behavioral recovery.


In five patients with traumatic brain injuries, we investigated somatosensory representation of the central and peripheral surfaces of the body, and the extent of central reorganization or "cortical plasticity." We administered functional magnetic resonance imaging (fMRI) pre- and post-injury. The in vivo functional anatomy of the corticospinal projections was examined by fMRI using a combination of the following techniques: 1) A non-standard "rediffusion" fMRI approach was applied using a high resolution echo-planar BOLD-sensitive sequence; 2) A T2* sensitive sequence with high spatial resolution was used in a single-slice version; 3) A T2-weighted sequence in a high resolution 3D volumetric version was used to identify the cortical landmarks; 4) A conventional T1-weighted spin-echo sequence was used to map the cytoarchitecture of the brain. The supratentorial cortical lesions were confirmed by brain imaging and neurosurgery. The patients were scanned pre- and post-injury and the results were compared. We observed a significant increase in the cortical areas which are involved in the control of the hand after injury. The changes in the cortical representation of the hand were observed in the parietal opercular region and in the frontal cortex. The results suggest that the functional anatomy of the somatosensory cortex in adults is plastic and can be modified by injury and rehabilitation.
583.9
COORDINATED SYNAPTIC AND ASTROCYTIC STRUCTURAL CHANGES IN RATS REARED IN COMPLEX ENVIRONMENTS. T.A. Jones*, N. Haerykay, and W.T. Greenough. Dept. Psychology and Neuroscience Program, Beckman Institute, Univ. of Illinois, Urbana, IL 61801.
Rats in a complex environment (EC) have larger neuronal dendritic fields and an increased number of synapses per neuron than do rats in a simple environment (SE). This is in comparison to animals housed individually (IC) or socially (SC) in standard laboratory cages. These neuronal effects are accompanied by hypertrophy and proliferation of astrocytes in the inferior olivary nuclei in layers I, III, and IV of the EC, and these effects were greater in the following months than in the following days of EC, IC, or SE housing. Stereological measures of the volume fraction and surface density of glial processes showed little change in these size categories following exposure to EC. However, there was a specific increase in the region of contact between glial processes and pre- and post-synaptic elements in EC rats, measured as the surface density of glial membrane in direct apposition to these synaptic elements. That is, glial processes become more enwrapping of synapses, an effect which may be indicative of greater involvement in synaptic activity and/or a response to elevated synaptic activity. The increased glia-synapse apposition bears a resemblance to the glial response previously reported in relation to increases in synapse efficacy (Wenzel et al., 1991). Ongoing work is assessing the possibility that structural and positional changes in presynaptic astrocytic processes occur in close temporal coordination with synaptic events. Supported by MH45321 and MH10422.

583.10
Astrocytes demonstrate dynamic experience-dependent growth and hence proliferative response in the visual cortex of rats reared in a complex environment compared to those reared in individual cages (Sirevaag and Greenough, 1991 Brain Res. 542:273-278). To further delineate the effect of experience on the growth and differentiation of astrocytes in the visual cortex, monocularly deprived rats were examined with GFAP.
Eye lid were saturated at P12. Saturated rats were assigned to one of two groups; those with sutures remaining in place until P80 (MD, n=6) and those that had the sutures removed at P75 and were allowed 5 days of light exposure (MD+/L+/n=6). A nonsensur control group P80 (CL, n=6) was used for comparison. Optical dissectors and random systematic sampling techniques were used to estimate the numerical densities (NV) of astrocytes, neurons and total glia. A stereological cytocrit intersection method was used to estimate the surface density (SGFAP) of GFAP immunopositive processes. The numerical densities and (SV) were estimated in layers 2/3, 4, 5, 6.
The NV of astrocytes was not significantly different between the three groups. Group MD had a significant (35%) decrease in the SV in layer 4 as compared to control. The ratio of SV to NV of neurons (SV/NV), an estimate of the amount of GFAP-immunoreactive astrocyte surface area per neuron, was significantly decreased in layer 4 in both monocularly deprived groups compared to control. These results suggest that early experience has a profound effect on the morphological characteristics of astrocytes in the mature brain. Supported by MH45321.

583.11
Rats given an optic nerve crush regain visual function despite a 90% loss of retinal ganglion cells. Thus, there is either restoration of information from retina to brain or there is an adaptation in that visual function occurs with less information transfer. The 2-[14C] deoxyglucose technique was used to determine local cerebral glucose use (LCGU) as an assessment of information transfer from retina to brain visual centers. Male and Long Evans rats (F344 x Wistar) were given a mild crush to the right optic nerve and LCGU was assessed 2, 9, or 22 days later. LCGU procedure was determined during stimulation with a flashing strobe-light and rotating bar pattern with or without psychostimuline (300 mg/kg, i.m.), a cholinesterase inhibitor known to activate retinocollicular pathways. Qualitative inspection of LCGU autoradiograms from rats 2 days after crush revealed a marked reduction in LCGU in the left superior colliculus, especially the superficial layer. There was only minimal restoration of LCGU at 9 or 22 days after crush, restoration was greatest in the portion of the superior colliculus adjacent to the brain midline. Quantification of LCGU according to the Sokoloff equation is in progress. These results provide evidence that visual function is restored after optic nerve crush in spite of a marked dysfunction in retinocollicular information transfer. Supported by BFMT 07 98 040.

583.12
Nitric oxide (NO) is a ubiquitous messenger in the brain. This cellular messenger has been shown to modify activity-dependent synaptic efficacy in hippocampal slices (Schuman & Madison, Science 245:1503-1506, 1991; O’Dell et al., Proc. Natl. Acad. Sci. USA 88:11258-11259, 1991). We are interested if NO is also involved in activity-dependent modifications of ocular-specific connections occurred in the visual cortex during the critical period for monocular deprivation. Eight kittens were monocularly deprived for 10 days starting at 44-46 days of age. Four of the kittens also received infusion of N-nitro-L-arginine methyl ester (NAME) into one visual cortex by an osmotic minipump while deprivation. At the end of the monocular deprivation, the ocular dominance of visual cortical cells was evaluated electrophysiologically. Our data showed that the ocular dominance histogram in NAME treated cortex shifted less than un-treated cortex. However, NAME did not completely abolish the effect of monocular deprivation. Preliminary results also showed that the effect of this NOS inhibitor on the ocular dominance shift was found in layers V-L but not in layer VI. Our results suggest that nitric oxide may play a role in modification of eye-specific connections in the visual cortex. However, the activity of nitric oxide synthase is probably not essential for the modification of the connections. Supported by EY 06474 and NS 29343.

583.13
We have recently demonstrated that inhibition of nitric oxide synthase by intramuscular injection of 30-150 mg/kg of the methyl ester (L-NAME) one hour before each training session blocks touch learning in Octopus vulgaris (Proc. Roy. Soc. London, B in press). We used discrimination between ~1 cm black plastic balls, one smooth and one rough as the learning paradigm. The balls could not be discriminated by vision alone but were readily distinguished by touch. We have now extended these studies to visual learning using discrimination between vertical and horizontal white rectangles as the learning paradigm. We have now begun a study of the effects on touch learning of intramuscular application of the NO synthase inhibitor L-NAME 10-9 to 10-5 mg/kg. After a few days this resulted in significant inhibition of touch learning. Since this inhibits heme oxygenase-2, the source of CO in brain tissue, it seems that CO, as well as NO, is necessary for touch learning. Wayne, Bonaventura and Sherm have recently shown that NO, at low concentrations, causes active extension of filopodia in neuronal cultures (Proc. Soc. Neurosci. 1993). We have shown that cytochalasin D, which also blocks filopodial extension, blocks touch learning in Octopus, so our findings suggest that filopodial extension may be essential to touch learning in Octopus. There is also some doubt when using drugs to inhibit learning as to whether the primary effect is on sensory or motor output rather than on the learning process itself. To address this question, we have shown that animals treated with L-NAME can recall a previously learned touch paradigm but cannot learn a reversal of the paradigm. This suggests that the drug acts directly on the learning process.

583.14
ABC AN ACTIVITY-REGULATED CYTOSKELETAL PROTEIN IS A BRAIN SPECIFIC IMMEDIATE EARLY GENE THAT ASSOCIATES WITH THE CYTOSKELETON. G.L. Lyford, K. Yamagami, W. Kaufmann, A. Lamanna, and C.A. Dancer* and F.F. Worley Department of Neuroscience, Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205 and JS, Division of Neuroscience, Memory and Aging, University of Arizona, Tucson, AZ 85724.
To define the molecular mechanisms of neuronal plasticity, we have identified novel proteins that are rapidly regulated by neuronal activity. In particular we have isolated a novel gene, Arc, which demonstrates co-localization and interaction with the neuronal cytoskeleton. Arc was isolated by differentiating axonal growth techniques from electrically stimulated hippocampus. The message encodes for a 33KD protein that is homologous to loop 21 and 22 of the structural protein, α-spectrin. Arc mRNAs and protein are rapidly and transiently induced in response to activity. Arc mRNA induction is not blocked by pretreatment with cycloheximide. Basal arc mRNA expression shows developmental regulation with levels rising at mid-late gestation and peaking at P16. Immunohistochemistry demonstrates Arc expression is restricted to neurons with protein detected in both cell soma and dendrites. Confocal images show that Arc co-localizes with the actin cytoskeleton in the sub-membranous cell cortex. In situ hybridization after seizure shows signal in both the cellular and membrane layers of the dentate. It is likely that arc mRNA is induced not only in the cell soma but also in the dendritic tree of the granule cells. Biochemical data corroborate the evidence from sequence homology and protein localization that arc is a cytoskeletal-associated protein. At the 90% arc mRNA induction from brain that greater than 90% of Arc immunoreactivity resides in the cytoskeletal pellet which is resistant to 10M HC1 and 1% Triton x-100 washes. We have further demonstrated that Arc co-localizes with actin cytoskeleton in the sub-membranous cell cortex. Together these findings suggest that arc is a cytoskeletal-associated protein which may play a role in dendritic structural changes underlying neuronal plasticity. This work is supported by EY03974 and AG09019.

We address the problem of fast reorganizational plasticity using a protocol of simultaneous, paired peripheral tactile stimulation (PPTS), motivated by the potential of Hebb that temporal coincidence of external events are required to evoke plastic change in the reorganization in the hindbrain representation of somatosensory cortex of adult rats, a few hours of PPTS induced a dramatic reorganization that included: 1) enlargement of receptive fields (RFs) that were comprised to the RFs of the stimulated sites. Quantitative analysis of response parameters confirmed the findings: 2) RF overlap was significantly between RFs representing stimulated skin fields 3) Cortical representational areas of the stimulated skin fields were several fold enlarged and were shifted by a siphid the hindbrain representational borders. 4) Response durations were elongated due to enhancement of late response components. 5) Computer-based reconstruction of somatosensory maps revealed distortions that were selectively related to the stimulated skin sites, but the overall topography was mostly preserved. 6) All effects were fully reversible after 6 to 8 hours after termination of PPTS. 7) Response amplitudes and latencies were not affected. The implications of residual plasticity for short term plasticity in representational maps of adults are now investigated in computer simulations based on self-organizing maps. These modifications of synaptic coupling without involving anatomical changes.

GENEERATION OF A SUBTRACTED cDNA LIBRARY FROM THE CEREBRAL CORTEXES OF AN ENVIRONMENTALLY ENRICHED RAT AND A STANDARD ENVIRONMENT RAT. S. J. Brooks, D. T. Ribeiro, M. C. Diamond, and J. J. Martinez J. Jr., Department of Integrative Biology and Department of Psychology, University of California, Berkeley, CA 94720

Environmental enrichment in rats, when compared to rats housed in a standard environment, induces a number of anatomical and structural changes in the cerebral cortex including: an increase in dendritic branching (Holloway 1966, Brain Res., 2:393-396), an increase in dendritic spine counts (Globus et al. 1973, J. of Comp. Physiol., Psychol., 82:175-181), an increase in glial cell counts (Altman et al., 1974, Neurosci. 2: 411-416), and an increase in the area of cortical RNA to unique rat RNA sequences (Uphouse et al., 1973, Dev. Psychobiology 2: 171-178). Together these results suggest that changes in gene expression underlie the enrichment-induced changes in the cerebral cortex. In an effort to identify some of the up-regulated genes, we performed a cDNA library subtraction using a ten-fold excess of standard cortex cDNA. We have begun characterization of the subtracted cDNA library and to date have isolated two genes, which are differentially expressed. Preliminary analysis indicates that these genes are differentially expressed. Further analysis will include sequencing the genes and In situ hybridization with the genes in the brains of both enriched and standard environment animals. Supported by Rennie Fund to Joe J. Martinez Jr.


The nigrostriatal dopaminergic system has been implicated in the behavioral neglect and recovery seen following unilateral ablation of the of the medial agranular area of prefrontal cortex (AAGm). In this study, immediate early gene (IEG) protein products was used to examine alterations in striatal neuronal response at 5 days (neglect group) and 21 days (recovered group) after unilateral AAGm ablation. Basal and dopamine agonist-induced (5 mg/kg d-amphetamine) c-fos levels in the striatum were examined. Adjacent brain sections were reacted with antisera to ZIF268 or Jun B, and the amount of immunoreactivity in the CPu was determined through image analysis. At 5 days postlesion, basal and AMPH-induced ZIF-immunoreactive (IR) nuclei in the ipsilateral CPu were fewer in number (25-30%, p<0.03) and smaller in size (6%, p<0.03) compared to those in contralateral CPUs. For Jun-IR nuclei at 5 days postlesion, no hemispheric asysemblies in basal levels were evident, while AMPH-induced levels were reduced by 25% in the ipsilateral CPus (p<0.03). This reduction was due to both a decrease in the number of nuclei (10%) of Jun-IR nuclei. For both Fos and Jun, hemispheric asysemblies were primarily restricted to dorsolateral CPUs, the region receiving dense afferents from AGm. In contrast to the 5-day findings, all hemispheric asysemblies were significantly diminished or not evident in recovered rats (21+ days).

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Unilateral removal of the medial agranular region of prefrontal cortex (AGm) results in behavioral neglect of contralateral stimuli followed by recovery in about 3 weeks. The present study used autoradiography to examine regional alterations in striatal NMDA receptors at 5 days (neglect group) and 21+ days (recovery group) after unilateral AGm ablation. Time-dependent asymmetries in [HGLU binding in the ipsilateral caudate-putamen (CPu) were decreased by 2% at 5 days postsurgery and increased by 9% at 21+ days postsurgery (p<.05). These effects were restricted to the dorsolateral CPu, the region receiving dense afferents from AGm. Contralateral lateral striatal asymmetry binding. These results are unlikely to be due to changes in CPu neuronal density following partial deafferentation since no lesion effects on [HThiC-253 binding were evident in adjacent brain sections. These findings indicate that a partial loss of corticostriatal afferents leads to an initial reduction followed by an upregulation of striatal NMDA receptor binding, perhaps as a compensatory response to the loss of GLU input. These NMDA receptor changes may be partly responsible for the behavioral neglect and recovery and for the time-dependent alterations of striatal immediate early gene expression seen following unilateral AGm ablation (see Marshall and Vargo, this volume).

584.6 NONCOMPETITIVE NMDA ANALYTIC AND ANTIDOPAMINANE DRUGS FACILITATE BEHAVIORAL RECOVERY FOLLOWING ELECTROLYTIC LESIONS OF THE RAT CORTEX. M.R. Hantsce, D. Smith, and M. Mark. Dept. of Psych., Texas Christian University, Ft. Worth, TX. 76129.

Following brain injury due to ischemia, hypoxia or fluid percussion trauma there is an extensive release of glutamate that opens a sequence of events leading to secondary cell death proximal and distal to the initial site of damage (i.e. excitotoxicity). These events are mediated by N-methyl-D-aspartate (NMDA) receptor activation. The ability of NMDA receptor activation to mediate excitotoxicity may be attenuated by neuropeptide drugs that either prevent or limit the activation of the N-methyl-D-aspartate (NMDA) receptor by glutamate or prevent the breakdown of the cell membrane by free radical scavengers). These neuropeptide drugs may facilitate behavioral recovery by limiting the extent of secondary brain damage. The present experiment investigated the possibility that neuropeptide drugs facilitate behavioral recovery following electrolytic lesions of the cortex. Three drugs were studied: MK-801 (a noncompetitive NMDA antagonist working at the PCP binding site); magnesium chloride (MgCl2; a voltage dependent noncompetitive NMDA antagonist); and N-tetra-hexyl-phenyliprione (PB; an antioxidant and free radical scavenger). Rats received the anesthetic sensorimotor cortex (SMC) and a regimen of MK-801 (1 mg/kg), MgCl2 1 mmol/kg), PBM (100 mg/kg) or saline beginning 15 min after the lesion. The rats were tested on a locomotor placing task. Saline treated animals showed the expected severe impairment in placing the contralateral forelimb during locomotion on the grid floor. In contrast, rats receiving, MK-801, MgCl2 or PBM showed a reduction in the magnitude of the initial deficit as well as an acceleration of recovery. These results suggest that neuropeptide drugs identified in models of ischemia and traumatic brain injury have the expected behavioral effects in an electrolytic lesion model. Moreover, they suggest a process similar to that of excitotoxicity may occur following electrolytic brain lesions causing transneuronal degeneration in areas distant from the lesion site.


There are several reports of dendritic reorganization following neural injury in adult rats. We have found that prefrontal, cingulate, and motor cortex lesions all lead to a chronic increase in dendritic arbor in parietal cortex ipsilateral to the lesion. Jones & Schallert (1992) also found increased dendritic growth in the normal hemisphere of rats with unilateral motor cortex lesions, and they correlated this with behavioral asymmetry. In contrast, we found that large unilateral strokes result in chronic changes of dendritic arbor ipsilateral to the lesion and no change in the normal hemisphere, in spite of a large behavioral asymmetry. The presence or absence of dendritic growth following these lesions may be due to differences in dendritic reactivity, to differences in motor behavior, or to differences in dendritic reorganization. We therefore made unilateral motor cortex lesions of varying sizes and at different ages throughout the lifespan and measured behavioral asymmetry. Animals were sacrificed at different postoperative intervals for GFAP, bFGF, vimentin, synaptophysin, and OX-42 immunohistochemistry, or Golgi-Cox analysis.

Dendritic change was related to age, the magnitude of the astrocyctic reaction, and lesion size, but we found no relationship between behavioral change and dendritic growth in the normal hemisphere.

584.8 TACTILE STIMULATION ENHANCES RECOVERY AND DENDRITIC GROWTH IN RATS WITH NEONATAL FRONTAL LESIONS. Bryan Kolb*, Grazyna Gorny and Robbin Gibb. Dept. of Psychology, University of Lethbridge, Lethbridge, Canada, T1K 3M4.

Rats were given medial frontal lesions or sham operations on postnatal day 4. Beginning on day 5 the animals were given 15 min of tactile stimulation with a small paintbrush three times a day until weaning at day 21. At 60 days the animals were trained in the Morris water task and the Whishaw reaching task. The behavioral results showed large behavioral deficits in lesion animals relative to normal control rats. These deficits were significantly attenuated by the stroking. At the end of testing the brains were processed for a modified Golgi-Cox stain or immunohistochemistry. Frontal lesions reduced brain weight and decreased dendritic reactivity, but there was no significant correlation. The tactile stimulation significantly reversed these effects, with the effects being larger in female than male rats. Spine density was decreased by tactile stimulation in both normal and operated rats. GFAP, OX-42, and synaptophysin immunohistochemistry is in progress. Overall, the results suggest that tactile stimulation during infancy may modulate the effects of perinatal cortical injury and may affect synaptic development in the normal brain.


It has been proposed that the cholinergic system plays a critical role in learning and memory (Deutsch, 1971, Science 174:788-794; Bartos et al., 1985, Ann NY Acad Sci 444:332-358). In support of this hypothesis are data demonstrating that cortical cholinergic hypofunction correlates with cognitive deficits in humans (Perry et al., 1978 Br Med J 1:149-151; Wolk et al., 1982 J Neurosci 2:427-439). In the present study, injections of choline into the basal forebrain have been used to further explore the relationship between cholinergic and behavioral function. Bilateral injections of coenzyme (3 ul AU/0.4 ul saline) or vehicle (0.4 ul saline) were made in the nucleus basalis magnocellularis (NBM) of male Long-Evans rats (n=12/group). Four weeks post-lesion, behavioral assessments were made for one-half of the rats in each group. Five weeks post-lesion, the rats were sacrificed, and ChAT activity was measured in several brain regions. The second half of the rats were tested behaviorally 11 weeks post-lesion and sacrificed twelve weeks post-lesion. Five weeks post-lesion, there was a significant decrease in cortical ChAT activity and passive avoidance cross-over latency and a deficit in the acquisition rate of a water maze task. Twelve weeks after cochlicine infusion, ChAT activity in the motor cortex of the lesioned side was not significantly different than vehicle-infused rats, although a significant decrease was seen in the passive avoidance cross-over latency. These data suggest an association between time-dependent changes and recovery of a water maze learning task. In contrast, the passive avoidance task had an equivalent deficit at both timepoints. In summary, these data show task-specific behavioral recovery associated with time-dependent recovery in a specific regional cholinergic marker.

584.10 BEHAVIORAL EFFECTS OF BILATERAL CORTEX LESIONS ON REACTION-TIME PERFORMANCE IN THE RAT. C. Bauer, P. Gulin, A. Nicollin and M. Amatric*. Cellular and Functional Neurobiology Laboratory, ENRES, 13402 Marselle cx 9 (France).

There is substantial evidence that suppression of cortical projections enhance striatal dopaminergic activity. Cortical ablation however does not result in obvious motor deficits on spontaneous behaviors. The effect of a bilateral cortical lesion upon reaction-time was thus studied in a conditioned motor task known to be sensitive to striatal dopaminergic activity. Rats were trained to depress and hold a lever until the presentation of a visual conditioned stimulus (CS) and then to release it with a reaction time (RT) of less than 500 ms for food reinforcement. Cortical lesions were performed by thermocoagulation of fronto-parietal areas. Such lesions are known to induce a progressive degeneration of the cortical layers. The animals were tested daily from day seven to thirty-five post-lesion. A dramatic short-lasting increase in the number of delayed responses (over 500 ms), recovering after 20 days, could be observed in the reaction-time task. This effect was classically considered as a motor impairment. In contrast, a significant increase in number of anticipated responses (premature release of the lever before the CS) was observed throughout the 35 days of testing. These results show that an extensive lesion of the fronto-parietal cortical areas induced a mixed effect with different time recovery. The long-lasting effect on anticipated responses could be related to the effects produced by a dopaminergic hyperactivity in the striatum. The short-lasting effect on delayed responses is suggested to result from damage of the direct motor pathways following extensive cortical lesions that are rapidly compensated by other mechanisms.
584.11 THE CONTRIBUTION OF TASK-SPECIFIC EXPERIENCE TO THE AMPLITUDE-INDUCED FACILITATION OF RECOVERY IN RATS WITH CORTICAL DAMAGE. T.D. Schmahmann, L.L. Bowerman, and T.M. Barth, Dept. of Psych., Texas Christian University, Ft. Worth, TX 76129.

In an early study it was shown that amphetamine facilitates the recovery of beam-walking after brain injury. However, no motor cortex damage (MCD) was only if the animals receive task-specific practice while under the influence of the drug. More recently it has been suggested that amphetamine and practice have independent effects on recovery of beam-walking. The present study evaluated the effects of amphetamine and practice on the recovery of tactile forepaw placing after unilateral MCD lesions. The behavior was chosen because amphetamine facilitates recovery from placing deficits even though the animals do not show placebo reactions during the time of drug intoxication. If task specific experience has a role in the facilitation of recovery it does not require the execution of the motor response while under the influence of the drug. 1) training with amphetamine (2 mg/kg) was given to six groups to effect recovery. Four groups were used: drug + practice (dp), drug - no practice (dp), saline + practice (sp), saline + no practice (sn). The dp group showed the fastest rate of recovery. Statistical analyses showed that there were main effects for drug and practice but no significant drug x practice interaction. These data suggest amphetamine and practice have independent effects on recovery of tactile placing. Two groups were selected to isolate the type of experience necessary to facilitate recovery of forepaw placing. Unilateral MCD damaged rats were given amphetamine and assigned to one of four different groups: practice on the contralateral side only, practice on the ipsilateral side only, hanting and no practice. The results were that rats receiving either ipsilateral or contralateral experience during the period of drug action recovered significantly faster than animals receiving no practice. These data suggest that task specific somatotopic stabilization on contralateral or ipsilateral control and motor experience on the task facilitate recovery of tactile placing.


Recent experiments revealed rapid reorganization of the cortical representations of the primary somatosensory area in young adult rats following intracortical (ICM) and intrathalamic (ITM) microstimulation (R1Casoni et al. 1992a; 1992b; 1993a; 1993b; 1994a; 1994b). While there is a substantial body of information on the reorganization at a cortical level, little is known about the nature of subcortical plasticity. The present study investigated the contribution of the thalamic ventral posterolateral nucleus (VPL) to the cortical reorganization elicited by ICMS and of the reorganization of the somatotopic representation in this thalamic nucleus induced by direct intrathalamic microstimulation (ITMS). The experiments were performed in adult rats weighing 180-220 g. Unilateral VPL neurons within the hindpaw representation in SP and in VPL were recorded with glass microelectrodes. Microstimulation was applied at a local cortical (ICMS) or thalamic (ITMS) site for 5-7 seconds. All experiments were recorded with the 18 Hz stimulation frequency. The data showed that ICMS and ITMS induced significant changes of VPL activity in the thalamus. While ICMS induced an increase of VPL discharge rates with no significant spatial changes in the skin representation, ITMS elicited changes in the somatosensory map at the VPL site. However, the changes are small compared to those described for ICMS, suggesting that this type of fast plasticity is a predominantly cortical phenomenon.

Suggested by Coordenadoria de Apoio ao Avanço de Recursos (CAPES-Brazil).


A fundamental issue in quantitative neuropathology is the extent to which processing of sensory information affects the complexity of dendritic trees. To study this, the basilar dendrites of supragranular pyramidal neurons in the human superior temporal and occipital association cortex (prefrontal, area 10) were compared with those in primary and secondary visual cortex. Neurons obtained from the left hemisphere of 5 neuropathologically normal subjects were fixed with a modified rapid Golgi technique. Quantification of 100 neurons (10 neurons per tissue block) was performed using the Neurolucida system (Microbrightfield, Inc.) according to accepted criteria (Jacobs & Scheibel, 1993, J. Comp. Neurol., 327, 83-96). Dependent measures were total dendritic length (DML), mean dendritic length (MDL), dendritic spine density (DS2), dendritic surface area (DSD), and total dendritic volume (TDV). A distinction was also made between proximal (1st, 2nd, and 3rd order) and the more distal portion of the dendritic tree (3rd order and above). The results indicated that in the prefrontal cortex, neurones in area 10 consistently exhibited higher TDL (19.8%), DSC (19.9%), and DS2 (11.6%) values than those in area 18. However, the overall distribution was similar between both cortical areas for TDL, DSC, and DS2, but distal segment MDL was an average of 7.6% greater than proximal segment MDL, which is consistent with Jacobs et al. (1993). J. Comp. Neurol., 217, 97-111). An order by order analysis of the dendritic envelope revealed that differences between the two cortical areas were most pronounced in 4th order dendritic segments. Significant age-related decreases in TDL, MDL, and DS2 were also noted. This finding of greater dendritic scropl in area 10 over area 18 provides tentative support in humans for the notion that dendritic system complexity reflects the computational demands placed on those systems. (Supported by grants from the National Science Foundation to DW and NS25742 to DFC.)

584.14 LEARNING INDUCED PLASTICITY OF CORTICAL MAPS - LACK OF CHANGES IN REPRESENTATIONS OF ADJACENT, UNSTAINED SENSORY RECEPTORS. M. Koonsnatt and S. Snulcik, Dept. of Neurophysiology, Neuroklinika, Naujoji Val. 34, LT-02133, VILNIUS, LITHUANIA.

Sensory training is known to produce an enlargement of cortical representations of receptors activated during the training (Jenkins et al. 1990; Rencane et al., 1992; Weisberger et al., 1993). Increase of cortical representations of trained receptors was accompanied by a decrease in extent of adjacent representations of receptors not involved in training. We have previously demonstrated that short lasting classical conditioning involving stimulation of vibrissa resulted in enlargement of vibrissal cortical representation. In the present experiment we examined if the enlargement of the trained row B of whiskers representation takes place at the expense of neighboring, untrained rows. During training stimulation of row B was paired with electrical irritation of the tail. Four pairings per minute were repeated for 10 min, a day for 3 days (altogether 130 pairings). A day after completion, 2-deoxyglucose mapping was performed, in which cortical representation of the trained row B and control row B on the other side of the snout were visualized. As we have shown previously, representation of the trained row B was enlarged by 45%, expanding into territories of row C and A. In another group of animals we examined if cortical representation of rows A and C bordering the trained row B, are narrower than usually. Analysis of 2DG autoradiograms revealed that labeling induced by stimulation of rows A and C is not decreased either in area or in intensity compared to control. The cortex corresponding to the trained row B representation (asundimented during 2DG mapping) showed higher labeling than on the control side. The results indicate that training-induced expansion of row B representation is a dynamic phenomenon, where regions of cortex are activated by a new input without loosing normal reactivity to the old one.

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The purpose of this study was to determine whether peripheral nerve grafts in the CNS can promote axonal regeneration resulting in behavioral recovery. Fourteen rats received bilateral knife-cut lesions of the fibria-fornix. Eight of the animals also received a sciatic nerve transplant placed between the septum and hippocampus. Four animals served as normal controls. Beginning 2 weeks after the transplant procedure, all animals were tested on a delayed-response alternation task and the Morris water maze. Testing continued for 2 months. Both experimental groups were significantly impaired on the spatial tests compared to the normal group throughout testing. Moreover, preliminary analysis of the results indicate that the sciatic nerve transplants had no facilitating effects on spatial performance.

Correlations between cholinergic innervation and behavior are currently being conducted.


585.5

RHEB, A RAS HOMOLOG ENRICHED IN BRAIN, INTERACTS WITH RAFI SERINE/THREONINE KINASE IN AN ACTIVITY-REGULATED MANNER. W. M. Yee1, A. Lanahan1, and P.F. Worley1. Department of Neuroscience, Sch. of Medicine, Baltimore, MD 21202.

It has been postulated that production of specific gene products in response to neuronal stimulation is required for the establishment and maintenance of neuronal plasticity. In our efforts to characterize activity-regulated gene products associated with neuronal plasticity and long-term potentiation (LTP), we have isolated and characterized Rheb, a novel Ras homolog enriched in brain that is induced by LTP and seizure stimuli and is regulated as an immediate early gene. Rheb is also induced by NGF, EGF, and FGF in neuronal cell lines and is enriched in the granule cell layer of the hippocampus following neurotransmitter stimulation. Rheb shares a high level of homology with human H-Ras and yeast RAS1 in conserved domains required for GTP-binding and hydrolysis, and appears to be a functionally close relative of human H-Ras as determined by Rheb's ability to transform mammalian cells.

In order to characterize Rheb's physiological role in neurons following neuronal stimulation, we have utilized the yeast two-hybrid system of identifying protein-protein interactions to isolate upstream and downstream interactors of Rheb. We report here that Rheb interacts with Raf1, a serine/threonine kinase required for the oncogenic activity of human H-Ras. Using an in vitro co-precipitation assay, we have confirmed that Rheb interacts with Raf1 kinase isolated from the hippocampus and cortex of postnatal day 21 rats. In addition, we have experimental evidence suggesting that the interaction between Rheb and Raf1 may be regulated by neuronal activity. This line of evidence suggests that Rheb may be acting as an activity-regulated "molecular switch" that activates Raf1 serine/threonine kinase following depolarizing stimuli or neurotransmitter binding.

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VARIABILITY OF POPULATION SPIKE AMPLITUDE IN LONG-TERM POTENTIATION IN BEHAVING RATS. S.J. Jones1, D. Moore2, and M.E. Corcoran3, 4. Dept. of Psychology, University of Victoria, POB 3050, Victoria, B.C., Canada, V8W 3P6.

Although long-term potentiation (LTP) has been postulated to be a mechanism of learning and memory, relatively few investigations of LTP have been performed in awake rats carrying chronic electrodes, and, of those, large variation in the amount of LTP has been observed across laboratories. To study factors influencing LTP, we measured the amplitude of the population spike (PS) evoked in the dentate hilus by stimulation of the medial thigh over the posterior paw in awake rats that had undergone hippocampal recording. In Experiment 1, LTP was induced in 1 group with high chemodenervation infusions of acSF, in another group with chemodenervation but no infusions, and in a third group with electrodes. The electrode group received greater gain than the chemodenervation group. Experiment 2 tested the effects of behavioral state on LTP. Data from 1 group were collected only when rats were immobile; data from group 2 were collected irrespective of ongoing behavior, and rats were in Experiment 3 tested the possibility that the saline injection may have suppressed LTP at 60 min after test, to mimic conditions of drug administration. No LTP was observed in either group at 60 min post-injection, whereas significant LTP was observed 24 hr post-injection. In Experiment 4, only group 2 exhibited significant LTP whereas both groups showed no LTP at 24 hr. In Experiment 4, 1 series of tetanic stimulations was applied each day for 9 days. Significant LTP was observed only after session 2 and asymptoted after session 6.

We conclude that the magnitude of LTP is affected by the presence of the chemodenervation and by prior injections and that induction of LTP is more reliable with multiple sessions of tetanization. (Supported by NISER)

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1Duke Univ. Med. Center, 2Harvard Med. School, 3Colombia University, 4Fukushima Medical College.

Fleeting changes of neuronal activity produce a lasting reorganization of synaptic connections in developing and mature mammalian nervous system. Activity-dependent interactions also contribute to a formation of abnormal synaptic connections in the mature nervous system. Expression of the immediate early gene, c-fos, has been postulated to link fleeting changes of neuronal activity to lasting modifications of neuronal function and structure in the mammalian nervous system. To test this hypothesis, we examined behavioral and electrophysiologic indices of kindling development and kindling-induced sprouting of hippocampal granule cell axons in wild type (+/+), heterozygous (+/-), and homozygous (-/-) mice null mutant of c-fos. Defects of both electrophysiologic and behavioral features of kindling development were evident in null mutants. Kindling-induction of granule cell axon sprouting measured by Timm staining was significantly attenuated in null mutants compared to +/+ mice with intermediate values evident in +/- mice. We conclude that c-fos is necessary for both normal development of kindling and complete expression of kindling-induced synaptic reorganization of granule cell axons. We suggest that the null mutation of c-fos produces this phenotype by altering seizure-induced transcriptional activation of target genes.

585.6

A NOVEL PROTEIN REGULATED BY SYNAPTIC ACTIVITY IN VISUAL CORTEX AND HIPPOCAMPUS. P. R. Brahman4, A. Lanahan5, and P.F. Worley1.

1Dept. of Neuroscience and Neurology, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21202.

A novel activity-regulated developmental immediate-early gene (Ar62) was cloned by differential screening of a rat hippocampal cDNA library. Ar62 encodes a brain-specific 6.5 kb RNA with a 186 amino acid active reading frame that is not homologous to known proteins. Sequence analysis predicts that Ar62 has multiple phosphorylation sites, but no glycosylation sites. Antisera raised against a bacterial fusion protein containing the 186 amino acid open reading frame recognizes a single 28 kd band in HEK 293 cells transiently transfected with an expression construct containing the open reading frame and a 28 kd doublet in rat hippocampus. This 28 kd doublet is rapidly induced in hippocampal follower seizure with a maximal induction at 4 hours. Fractionation of hippocampal tissue shows that Ar62 is primarily cytosolic, but not nuclear, and also is associated with the membrane fraction. Northern blot analysis shows that Ar62 is induced in hippocampus within 30 minutes following seizure and is super-induced by pre-treating with cycloheximide. In situ hybridization studies show that Ar62 is strikingly inducible in visual cortex. Exposing dark-reared rat pups to light dramatically increases Ar62 mRNA in visual cortex, and unilateral intracocular injection of TTX reduces basal expression of Ar62 in the contralateral visual cortex. A role for Ar62 in synaptogenesis is suggested by the redistribution of Ar62 message in cortex during a critical period of exophytic synaptogenesis in the second post-natal week in rats. The distribution of Ar62 changes from a diffuse cortical distribution in day 10 rats to a specific localization in cortical layers II and V in day 14 rats. In addition, Ar62 is strongly induced in dentate gyrus in an NMDA-dependent manner following a high frequency LTD stimulus. Ar62 is a novel protein regulated by synaptic activity in cortex and may be involved in cortical synaptogenesis. Supported by EY08547.

585.8

DEHYDROEPiANDROSTERONE SULFATE INCREASES PS IN DENTATE GRYUS LTP IN INACT RATS. A. Yoo1, J.Harris2, and B.Bubryowa3. Dept. of Physiology, McGill University, Montreal, Quebec, Canada H3A1L1.

Dehydroepiandrosterone Sulfate (DHEAS), a neurosteroid secreted by both the adrenal cortex and the glisa, has been shown to behave as a non-competitive antagonist of the GABA receptor which exerts a inhibitory effects on LTP development when activated. Thus, it could be hypothesized that DHEAS would enhance LTP formation. Experiments were performed in intact anesthetized rats (1,5mg/kg). Electrodes were stereotaxically positioned in the perforant path (bifocal for stimulation) and in the dentate gyrus (monofocal for recording). DHEAS (20 mg/kg) was injected in the femoral vein, dissolved in Nutralipid 10% which served as control injection. Our data showed that after hormonal treatment primary tetanic stimulation (8 trains of impulses, total duration of 1 sec, train rate of 0.03 Hz, frequency of 350 Hz and pulse duration of 500 microsec each half wave), the amplitudes of the population spike (PS) increased approximately 2 fold over the ones from control experiments. In contrast, slopes of EPSP were shifted towards shorter durations with the hormone. The results, consistent with the work of Heyer et al.(1993) in CA1 area of rat hippocampus, suggest that hormonal compounds could differentially affect selected neuronal loci.
585.9
REWARD-SCHEDULE EFFECTS AND GRANULE CELL NEUROGENESIS IN THE DENTATE GYRUS OF THE RAT
G.L. Eppig* , A. Avoli† , J.A. Gage . Dept. of Psychology & Inst. for Neuroscience, University of Texas, Austin, TX 78712
Granule cell neurogenesis in the rat dentate gyrus has been shown to continue well into adulthood, although peak neurogenesis occurs at the end of the first postnatal week. We recently examined the effect of a number of kinds of learning on granule cell neurogenesis in 11-12-day-old pups (Eppig & Avoli, Soc. Neurol. Neurosci., Abst., 1993). No significant training effect was found, but there was a significant effect due to handling. The experiment has now been replicated with 17-18-day-old pups. The pups were trained for 5 sessions (3 session per day). 30 trials per session. There were 3 groups defined by reinforcement schedule: continuous reinforcement, partial reinforcer, or reinforcer in a pattern of single alternation (PSA). A hippocampal stimulation training, but was put through all other specific aspects of handling in the training situation. A fifth group was an unhandled control: no handling and no training. At the end of each of the 2 training days, pups from all groups were injected intraperitoneally with Methyl-β-HTHThymidine (activity 2Ci/mmol, SmCign w/v) in order to label newly forming cells. Coronal brain sections were coated with Kodak NTB-2 emulsion, exposed, developed, and then counterstained with cresyl violet. Computer-assisted counts revealed no significant differences among all five groups including no handling effect. At 17 days of age, rat pups can learn PSA only at an 8-second ITI. To test the possibility that neurogenesis of granule cells in the dentate gyrus is influenced only at long ITIs, the first 11-17 day-old experimental groups are being replicated with longer ITIs. Data are also being collected on the effects of postnatal handling administered by artificial rearing from days 4 - 9. Supported by NIAAA grant AA07052.

585.10
Growth-associated protein-43 (GAP-43) is a nervous system-specific phosphoprotein whose expression is correlated with axon growth during regeneration and development. We are interested in the involvement of GAP-43 in axon elongation and branching in the developing nervous system. Using a model of selective ablation of the dentate gyrus (DG) of the hippocampus, and subsequent growth and reorganization of the denervated areas by other afferents projecting to the DG. Preliminary data suggest that the sprouting response induced by electrolytic and aspiration methods may be qualitatively and quantitatively different. While under nebulized anesthesia (50 mg/kg), the right EC is lesioned (aspiration or electrolytic) in male Sprague-Dawley rats. The animals survive for 2, 6, 15, or 30 days before sacrificing. The brain is removed, embedded, sectioned, and then immunoreacted with GAP-43 or GFAP antibodies. Following electrolytic lesions of the EC, the inner molecular layer of the DG expands to a greater width than following aspiration lesions as evidenced by GAP-43 immunoreactivity of brain sections. In addition, astrocytes are more immunoreactive with GFAP antibodies indicating a more robust gliosis following electrolytic lesions than after aspiration lesions. We are currently investigating other differences between the two types of lesions in generating a sprouting response in DG. We postulate that in DG, the various types of nerve cells discharged during the electrolytic lesion may recruit anionic pathways and the ECM utilizes a variety of mechanisms to reorganize the DG.}

585.11
SYSTEMIC ADMINISTRATION OF NALOXONE AFFECTS EPSP-SPIKE COUPLING IN LONG-TERM POTENTIATION OF THE LATERAL PERFORANT PATH. R.D. Kirkby*, C.B. Bramham, and J.M. Sarvey. Department of Pharmacology, Uniformed Services University, Bethesda, MD 20814
Previous research suggests that activation of opioid receptors is necessary for the induction of long-term potentiation of field responses evoked in the dentate gyrus by electrical stimulation of the lateral perforant path, the terminals of which may release both glutamate and opioid peptides. To further assess the role of opioid receptors in long-term potentiation in this pathway, we systematically administered naloxone (10 mg/kg; i.p.) 60 min prior to tetanic stimulation of the lateral perforant path in urethane-anesthetized adult rats. Tetanic stimulation consisted of 10 trains (60s intertrain interval) of 8 pulses (200 Hz; 400 Hz).
Contrary to expectation, naloxone- and saline-treated rats demonstrated similar increases in the initial slope of the excitatory postsynaptic potential (EPSP) recorded in the hilus. On the other hand, the magnitude of the population spike as a function of the slope of the EPSP after tetanus was somewhat smaller in rats treated with naloxone. The findings may shed light on the participation of opioid receptors in synaptic plasticity in the lateral perforant path. Supported by NIH NS32885.

585.12
We present a kinetic model of associative Long-Term Potentiation (LTP) in the hippocampus. We used an extension of a previous work on simulation of LTP (M.Ayala and G.F.Ayala, Neur. Comp. 5, 103 (1993)) to implement the two populations of synapses activated by a weak (W) and/or a strong (S) afferent pathways to the same hippocampal dentate region. The model was fine tuned with good agreement with experimental data on the associative properties of LTP and its modifications when using different protocols of stimulation. The model suggests a possible interpretation of the experiments in terms of molecular processes and a possible key role of retrograde messengers in the induction of associative LTP. In particular, the model suggests that the associative properties of LTP could be explained in terms of the modulating effects by the retrograde messengers on the coupling mechanisms between the W and S pathways. Two possible modes of coupling the two pathways have been tested with this model: one pre- and one post-synaptic, and both are in good qualitative agreement with experimental data.

585.13
We propose a kinetic model that suggests an interpretation of experiments in terms of the molecular processes that control the induction and maintenance of Long-Term Potentiation (LTP) and Long-Term Depression (LTD). The model suggests that LTD and LTP could be maintained by two similar but distinct autocatalytic processes activated by the same class of retrograde messengers. In particular, we propose that these two processes could interfere with the normal synaptic transmission mechanisms, increasing (LTP) or decreasing (LTD) the amount of neurotransmitter released at each stimulus. We present simulations of the effects of application of inhibitory retrograde signal production such as L-NAME and ZnPP, and application of Nitric Oxide scavengers, such as Hemoglobin. Simulations' results suggest an explanation of the experimental findings that retrograde messengers are infiiuent to maintain LTP or LTD, whereas an appropriate level of retrograde signal is needed to induce or maintain LTD and/or LTD during tetanic stimulation.

585.14
We have previously demonstrated changes in the hippocampal immunoreactivity for mAChRs and PCKy after spatial learning (Van der Zee et al., J. Neurosci., 1995). In the present study we determined whether 500 msec trace eyeblink conditioning induces changes in mAChR- and PCKy in the hippocampus. Young adult (3 months) female NZW rabbits were trace conditioned (3x2) and behaviorally naive animals (n=8) served as controls. 24 Hrs after a rabbit reached the 80% criterion or after the 15th day of training, the animals were transectedally perfused with 2.5% paraformaldehyde + 0.025% glutaraldehyde. Free-floating sections were immunostained for mAChR- and PCKy. In the hippocampus, only moderate levels for mAChR- and PCKy were observed for mAChR- and PCKy in control animals were determined to be the same in naive and trace-avoided animals. One animal only reached 40% CR's showed similar changes for mAChR- and PCKy, whereas the conditioning animals showed a variable effect for mAChR- and PCKy. These results demonstrate protein-selective changes in the hippocampal principal cells in trace eyeblink conditioned rabbits which may underlie the accompanying behavioral and electrophysiological changes. (Supported by the Netherlands Organization for Scientific Research (NWO) to E.A. and by NYU R01 MH47340 and R01 AG058796)

Neurofilaments (NF) are cytoskeletal proteins which play a fundamental role in neuronal morphology and axonal transport. Enhanced neuronal connections are formed during learning. However, it was previously reported in relation to learning and memory in the brain. In the present study we determined whether the immunoreactivity (ir) for the low subunit NF changes in the hippocampus during acquisition of trace eyeblink conditioning. A hippocampus-dependent task. Young (3 months) female NZW rabbits were trace conditioned (n=8), Pseudoconditioned (n=8) and behaviorally naive animals (n=6) served as controls. 34 hrs after the rabbits reached the 80% criterion, or was trained for 15 days, the animals were transcardially perfused with 2.5% paraformaldehyde. Free-floating cryostat sections were immunolabeled with a phosphoprotein-dependent (88kD. Sigma. NR4) or a phosphoprotein-dependent (NDK. Chemicon. MAB1615) anti-NF antibody. No gross changes in staining-intensity were found for either antibody in the principal cells or interneurons between the different groups. However, trace conditioned rabbits showed a twofold increase in fiber density for NR4 in the stratum oriens, and approximately a 50% decrease in the number of fiber crossings for MAB1615 was found in the stratum radiatum as compared to pseudoconditioned and naive animals. The increase for NR4-ir most likely results from an increase in NF-contents in CA1 output fibers. The decrease in the MAB1615-positive fibers most likely indicates an increase in dephosphorylation allowing the assembly of NFs formed from the low-subunit, and enabling the neurons to reshape their axons. These results indicate region-selective changes in the hippocampus of trace eyelink conditioned rabbits which may underlie learning and memory processes. (Supported by the Netherlands Organization for Scientific Research (NWO) to E.A.V.d.Z. and by NIH ROI M014730 and ROI AI00798)

585.17 BRIEF TETANIC STIMULATION CAUSES SELECTIVE TRANSIENT INHIBITORY SUPPRESSION IN THE DENTATE GYRUS N. W. Migram* and J. Ferbinteanu University of Toronto, Scarborough Campus, 1265 Military Trail, Scarborough, Canada, M1C 1A4.

Several studies have reported that repeated activation of hippocampal afferents leads to a loss of recurrent inhibition. These findings suggest that inhibition is susceptible to use dependent breakdown. To further understand the conditions necessary for such an inhibition, we have investigated the effect of brief stimulus trains to the perforant path on both paired pulse inhibition and facilitation in the dentate gyrus with urethane anesthetized male hooded rats. An afferent pulse procedure with a reference pulse, a variable intensity conditioning pulse (C-pulse) and a test pulse (T-pulse) was used. The interval between the C and T pulse was set at 25 msec and the intensity of the T pulse was held constant. Under the control condition, there was paired pulse facilitation with a low intensity C pulse, while a high intensity C pulse caused paired pulse inhibition. Trains with 5 pulses were applied at frequencies of 2 Hz 200 Hz, 15 seconds before delivery of the T pulses. The paired pulse facilitation, a loss of both paired pulse facilitation and inhibition. Increasing the number of pulses to 17 at a 2 Hz frequency had no incremental effect on paired pulse suppression, but attenuated the loss of paired pulse facilitation. There was evidence that the inhibitory loss was transient, since it was reduced when the delay between the trains and the T pulse was set at 1 minute. Work currently in progress is examining whether this use dependent inhibitory loss is enhanced by treatments which induce epileptic seizures.


Neurobiological signals, such as the EEG, are usually nonstationary over time. One way to account for the temporal nonstationarity of a signal is to use time-frequency analysis to reveal its time-frequency relations. In this report, the short-time Fourier Transform (STFT), an extension of the Fourier Transform, has been used to analyze the EEG recorded from entorhinal cortex (EC) and dentate gyrus (DG). The resulting 2-dimensional time-frequency spectrum image was obtained via a 2A sliding window with a 1.9s overlap. Thirty second samples of EEG (sampled at 256 Hz) were extracted simultaneously from the EC and the DG of behaving rats. During the middle 10s of each 30s epoch, a low intensity train of electrical pulses at one of 5 frequencies (2.610, 14,181Hz) was applied to the pyriform cortex (PC), which projects to the EC. The EC projects, in turn, to the DG. Although there was no or little visually apparent change in the EEG produced by the stimulation, the time-frequency spectrum image showed clear peaks at the stimulation frequency and its harmonics. The time-frequency form of the coherence function was calculated based on the STFT (15 EEG epochs) to study the connectivity between the EC and the DG. Coherence was high for the theta frequency, which fluctuated around 7-9 Hz during the 30s sampling period, and was also enhanced during stimulation at the stimulation frequency and its harmonics. The STFT was also used in signal synthesis (or extraction). The portion of the time-frequency image at the stimulation frequency and its harmonics (1-2Hz) were extracted and an inverse Fourier transform was performed on the extracted signal. The effect of the stimulation train, originally embedded in the raw EEG, showed up clearly in the reconstructed signal. Our results indicated that the STFT is an effective technique for signal processing of the nonstationary EEG.


Aged rats can be potentiated to the same degree as young ones by proper path stimulation, but they lose their potentiated response in the dentate gyrus more rapidly (Barnes, J. Comp. Physiol. Psychol., 1979, 93: 74; de Toledo-Morrell et al., Neurobiol. Aging, 1988, 9: 581). We showed earlier that the induction of LTD in the dentate gyrus of young rats is followed by a selective increase in the number of axospinous synapses with multiple, completely partitioned transmission zones (Geinisman et al., Hippocampus, 1993, 3: 455). To determine whether structural synaptic plasticity induced by LTD in aged animals is similar to that observed in young adults, aged (27-31 mo.) F344 rats were implanted with stimulating electrodes in the medial perforant path and recording electrodes in the hilus of the ipsilateral dentate gyrus. Potentiated rats were stimulated (with fifteen 20 ms bursts at 1 Hz) delivered at 0.5 Hz on each of 4 consecutive days and sacrificed 1 h after the fourth stimulation. Aged animals did not differ significantly from young controls in terms of the extent of potentialization. The number of synapses per neuron was differentially estimated for various synaptic subtypes in the middle (MML) and inner (IML) molecular layer of the dentate gyrus using the unbiased dissector technique. Only axospinous synapses with multiple, completely partitioned transmission zones were significantly increased in numbers in the MML, but not in the IML of potentiated aged rats relative to their stimulated (at a frequency of 0.2 Hz) or implanted controls. The magnitude of this change was practically the same in old and young responsive controls. This finding suggests that the observed structural synaptic modification may account for the equivalent extent of potentiation in rats of different chronological ages. Supported by Grants AG08794 from NIA and BNS-8912372 from NSF.

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586.1


Following complex motor learning, rats exhibit an increase in the number of synapses/neuron in the cerebellar cortex. This experiment examined which synapse types are altered. Female rats, approximately 8 months of age, were randomly assigned to an Acrobatic motor learning Condition (AC), a Forced Walking Exercise Condition (FW), or an Inactive Condition (IC). AC animals were trained for 30 consecutive days on a motor learning task which consisted of a series of obstacle laden pathways requiring a significant amount of motor coordination to complete. The WX animals were housed with running wheels attached to their home cages to which they had unlimited access and the IC animals were housed alone. The rats were shifted to a daily handling schedule equivalent to that experienced by the other groups. The density of synapses was obtained using the physical disector on serial electron micrograph montages taken within the molecular layer of the cerebellar cortex and synapses were classified as to their pre and post synaptic origins. Purkinje cell density was obtained using the physical disector on serial 1 μm sections of the cerebellar cortex and data were expressed as synapse/Purkinje cell. Results indicate that the AC animals have a greater number of parallel fiber to Purkinje cell synapses in comparison to controls. Detailed assessment of other synapse subtypes is in progress. Supported by AG 10154, NSF BNS 88 21219 and NSERC.

586.2


Following complex motor learning, rats exhibited increased numbers of synapses/neuron in the cerebellar cortex. Here we present preliminary findings examining the structural plasticity of the parallel fiber following motor skill acquisition. Female rats were randomly assigned to an Acrobatic motor learning Condition (AC), a Forced Walking Exercise Condition (FW), or an Inactive Condition (IC). AC animals were trained for 30 consecutive days on a complex motor learning task which consisted of a series of obstacle laden pathways requiring a significant amount of motor coordination to complete. The WX animals were housed with running wheels attached to their home cages to which they had unlimited access and the IC animals received the same handling schedule as the AC and FW rats. The intervariocyst distance along Golgi impregnated parallel fibers did not significantly differ among the three groups suggesting that few new pre synaptic varicosities had developed. The second experiment utilized the same behavioral paradigm and examined the number of single varicosities forming multiple synaptic contacts (double synapses) using unbiased stereological measures from serial electron micrographs. AC rats had significantly more double synapses/Purkinje cell than WX or IC rats. The increase in synapses in the cerebellar cortex following motor skill acquisition appears to involve a reorganization of parallel fiber varicosities. Supported by AG 10154, NSF BNS 88 21219 and NSERC.

586.3


Following training on a complex motor learning task, structural changes have been observed in the rat motor cortex (across adjacent posteriors). Previous research has also shown Fos to be expressed during learning. In this study, the expression of Fos was examined during the acquisition and maintenance phases of training on a complex motor learning task. Female rats were randomly assigned to an Acrobatic motor learning Condition (AC), a Forced Walking Exercise (FW) or an Inactive Condition (IC). AC animals were trained for 30 days on an elevated runway equal in length to the acrobatic course which required a substantial amount of motor skill to traverse. Each AC animal was pair matched with a WX animal which was forced to travel a flat elevated runway equal in length to the acrobatic course. The WX animals received no motor training or activity but were handled daily. Five animals from each condition were sacrificed after 1, 2, 5, 10 and 20 days of the experimental manipulation. Four 100 μm coronal sections containing motor cortex (1.6 mm to -1.4 mm from Bregma) were taken from one hemisphere of each animal and immunohistochemically stained for Fos. Sections were analyzed for the percent area stained for Fos. Using the disector method, the number of Fos positive cells was counted and expressed as a percentage of the total number of neurons in each condition. The results indicate that the AC animals have a greater percentage of Fos positive cells during the acquisition phase (days 1, 2 and 5) versus the maintenance phase (days 10 and 20) of training. Fos expression was not significantly changed as a function of time in the WX and IC animals. Supported by AG 10154, MH 40631, NSF BNS 88 21219 and NSERC.

586.4


Following training on a complex motor learning task, changes have been observed in synapse number in the rat cerebellar cortex. In this study, the number of synapses per neuron was examined in Layer II/III of the motor cortex following training on a complex motor learning task. Female rats were randomly assigned to an Acrobatic motor learning Condition (AC), a Forced Walking Exercise (FW) or an Inactive Condition (IC). AC animals were trained for 30 days on an elevated runway equal in length to the acrobatic course. Each AC animal was pair matched with a WX animal which was forced to travel a flat elevated runway equal in length to the acrobatic course. The WX animals received no motor training or activity but were handled daily. Five animals from each condition were sacrificed after 1, 2, 5, 10 and 20 days of the experimental manipulation. Ten 200 μm coronal sections were taken through one hemisphere (1.6 mm to -1.4 mm from Bregma) from each animal. Blocks of tissue containing the motor cortex were removed and embedded for electron microscopy. The physical disector was used to obtain neuronal density from 80 serial 1 μm sections through Layer II/III of the motor cortex. Synaptic density was measured using the physical disector on 16 serial electron micrographs taken within Layer II/III. The number of synapses/neuron in each animal was estimated from these two measures. The AC animals had significantly greater number of synapses/neuron than the WX and IC animals. Supported by AG 10154, MH 40631 NSF BNS 88 21219 and NSERC.

586.5

VISUALLY-INDUCED PLASTICITY IN THE AUDITORY SPACE MAP OF ADULT BARN OWLS. M. S. Brainard* and E. J. Knudsen. Dep. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305-5401.

The optic tectum (superior colliculus) contains mutually aligned neural maps of visual and auditory space. Prior experiments have demonstrated that during development and the topography of the auditory map is calibrated by visual experience: in owls reared with prismatic spectacles that optically shift the visual field along the along the horizon, the map of auditory space in the tectum gradually shifts to become aligned with the optically displaced visual map. In this study, we examined the capacity of the auditory space map in adult prism-challenged owls to recover normal topography following restoration of normal visual experience.

Owls were reared from shortly after eyelid opening (16-19 days) with prismatic spectacles that displaced the visual field by 2 degrees to the left or the right. After owls reached adulthood, the tectal auditory space map was assessed for changes induced by exposure to dichotically presented stimuli of varying interaural time difference. For owls in which visual experience had been restored and the tectal auditory space map, prisms were removed at ages ranging from 300 to 2000 days, and subsequent changes in the auditory map were assessed.

In all groups, regardless of the age at which normal vision was restored, the tectal map of auditory space recovered essentially normal topography. This recovery occurred rapidly, over periods ranging from 10-30 days. The results indicate that substantial visually-induced plasticity in the tectal representation of auditory space is maintained in adult owls. This finding contrasts with the plasticity observed in the visual and somatosensory domains which have suggested a loss of plasticity in the tectal map of auditory space and in sound localization behavior beyond a critical period in development.

Supported by NIH R01 DC 00155.

586.6


The effects of soiled bedding on synaptic morphology in the accessory olfactory bulb (AOB) were examined in adult male rats. Forty-day-old male rats were isolated. One group was exposed to bedding soiled by male and female rats (EC). Another group was only male soiled bedding (SC). The last was exposed to clean bedding (FC) for 2 months, the animals were sacrificed for electron microscopy. The size and the numerical density of synapses were measured in the granule cell layer and the granule cell layer. In the granule layer, the length of the synaptic contact zone was significantly greater in the EC than in the SC and the IC, while there was no statistically significant difference in the density of synapses among the three groups. Two types of synapses were classified in the granule cell layer (1) perforated synapse and (2) nonperforated synapses. For perforated synapses, the length of the synaptic contact zone was significantly greater than normal owls, regardless of the sex of the rat. For nonperforated synapses, there was no statistically significant difference in the length of the synaptic contact zone among the three groups. In the density of both perforated and nonperforated synapses, there was no statistically significant difference among the three groups. These results demonstrated that exposure to bedding soiled by female rats, which contains female odour substances (pheromones) can induce structural changes of the synapses in the AOB of male adult rats.
586.7
THE AT-14 mRNA IS ENRICHED IN SEVERAL AVIAN SONG CONTROL NUCLEI AND ENCODES AN RCS/NEUROGRANIN-RELATED PROTEIN S.M. Siegert* J.M. George, H. Jin and D.P. Clayton* Dept. of Cell & Structural Biology, University of Illinois, Urbana, IL 61801

Differential CDNA hybridization experiments led to the identification of a brain-specific, forebrain-enriched RNA (HAT-14) that is especially abundant in song control nuclei HVC and IMAN of canaries and zebra finches. The sequence of the HAT-14 cDNA predicts a 73 amino acid protein that contains a domain found in the GAP-43 and RCS/neurogranin proteins, which it is believed to encode protein kinase C-regulated calmodulin binding. Further analysis of the sequence reveals the first 50 amino acids are 84% identical between rat RCS and HAT14. The identity drops to 25% in the remaining C-terminal portion, with lack of conservation of the residues identified as "collagen-like" in the rat sequence. Antibodies raised against predicted HAT-14 peptide sequences react with neurons in the songbird telencephalon. In sharp contrast to RCS/neurogranin (which is not expressed outside the telencephalon), high levels of HAT14 expression are also observed in cerebellar Purkinje cells in the songbird. Supported by NIH Grant NS-25742.

586.8
DEVELOPMENTAL REGULATION OF GAP43 MRNA IN AVIAN SONG CONTROL NUCLEI. H. Jin, H. S. Simpson, S. Siegert, C. Mello, M. Iuculano, K.L. Neukirch, J.M. George and D.P. Clayton* Dept. of Cell and Structural Biology, University of Illinois, Urbana, IL 61801

GAP-43 is an axonal protein whose expression increases during neurite outgrowth, and which undergoes phosphorylation by Protein Kinase C during long-term potentiation. The mRNA for GAP-43 is proposed to be involved in both neural development and adult plasticity. The avian song control system stands as one of the best models of regulated neural plasticity. To determine whether GAP-43 may regulate plasticity in the song system, we cloned the cDNA homolog of the GAP-43 cDNA. We used this cDNA to measure in situ hybridization the expression of GAP-43 mRNA in the adult canary brain, and in the zebra finch brain during the critical period for song system development. These studies have emerged. First, GAP-43 is not notably abundant (compared to surrounding telencephalon) in the principal song nuclei HVC and RA in adult songbirds, but is relatively upregulated in nucleus N18TG2. Second, GAP-43 mRNA levels remain relatively stable throughout zebra finch development (15-110 days of age) in most song nuclei, with one exception: RNA levels are increased in nucleus RA when the bird is first learning to sing (before day 60) then fall to the low levels observed in adults. These results suggest GAP43 gene regulation does not play a significant role in the initial establishment of connections within the song control circuit, although the gene is expressed constitutively and thus the protein itself may serve a function. In RA, downregulation of the gene may be correlated with stabilization of the motor control pathways for song production.

(Supported by NIH grant NS-25742)

586.9

Gonadal steroids influence performance on a variety of spatial tasks in both humans and rodents. In estrogen modifies the structure and response properties of neurons in the hippocampus, an area believed to be involved in processing spatial information. The present study examined the effects of estrogen on spatial performance and various measures of hippocampal physiology. Ovariectomized rats with and without hormone replacement (estradiol-progesterone regimen) were used to mimic the natural cycle and control rats at various stages of the estrous cycle were tested in the Morris water maze and spontaneously firing neurons in the CA1 region of the hippocampus was then assessed in vivo at the same stage of the estrous cycle as at behavioral testing. A gradient of effect of hormones was observed for swim task performance and potentiation following primed burst stimulation: High levels of circulating estrogen related to poorer swim task performance and to higher levels of primed burst potentiation. Thus, a significant negative correlation between spatial memory and magnitude of synaptic plasticity emerged. We are presently examining the effects of fluctuations in estrogen on sleep and other behavioral measures in female rats. Possible actions of circulating estrogen on hippocampal function include: 1) neuromodulatory effects, via hippocampal afferents 2) direct, activational effects on hippocampal function and 3) regulation of number and/or function of synapses. Supported by AG00540 (DLX), AG07648 (PEG) and NS31830 (TFC).

586.10

We investigated whether the TH gene is expressed in the SN and whether it contributes to the motor symptoms of SN-P. TH mRNA was observed in the nigrostriatal system, the periventricular tegmentum, substantia nigra, and the red nucleus. We used the TH gene as a probe to examine the neuronal phenotype of SN-P in the rat. Sustained rotational behavior was observed following injection of the TH reporter gene construct into the SN-P nigrostriatal system. We have demonstrated that the TH reporter gene construct can be useful for identifying and targeting TH expressing neurons within SN-P. The gene transfer strategy used in this study may provide the basis for understanding the cellular mechanisms underlying the motor symptoms of SN-P.

586.11
NIGROSTRIATAL PROJECTION AFTER UNILATERAL 6-HYDROXYDOPAMINE INJECTION INTO THE STRIATUM IN NEONATAL RATS. Y. Ichitan1, M. Takagami and T. Konishi,1 Inst. of Psychology, Univ. of Tsukuba, Tsukuba, Ibaraki 305, Japan.

In order to investigate the reorganization of nigrostriatal dopaminergic neuron system after a lesion of this system, we examined the effects of neonatal 6-hydroxydopamine (6-OHDA) injection unilaterally into the striatum on the subsequent development of contralateral projections from the intact side of substantia nigra (SN). After treatment of dopamine levels was (20mg/kg, s.c.), rats received unilateral injection of 6-OHDA (10mg/5pl) into the striatum on the day 2 after birth. At the age of 4 weeks to 5 months, cholera toxin B subunit (CTB, 1%) was injected (0.4pl) into the striatum in order to label the CTB-immunoreactive cell bodies in the SN contralateral to the injection regardless of the age of animals. Results suggest that unilateral lesion of the neonatal nigrostriatal system did not have marked effects on the formation of interhemispheric connections of this system.
586.13
DEVELOPMENTAL CHANGES IN THE VESTIBULO-OCULAR PATHWAYS DURING METAMORPHOSIS IN FLATFISH. J.K. Jansen* and P.S. Eger. Dept. of Physiology and Institute of Biology, University of Oslo, N-0316 Oslo, Norway.

During the first two months after hatching, flounders go through a very unusual metamorphosis. They tilt their bodies progressively to one side while the one eye migrates in the medio-lateral direction across the dorsal midline to settle next to the other eye, on the opposite side of the head. During this process the vestibulo-ocular pathways are presumably reorganized to compensate for the right-left disalignment of visual and vestibular frames of reference. In the present study we have examined the vestibular nuclear complex with anterograde and retrograd neuronal tract tracing techniques (HRP and dextranamine) before and after the period of eye migration in larvae and juvenile turbot.

We find: 1. that the vestibular complex consists of spatially segregated groups of neurons selecting distinct pathways to reach their targets in rostral eye motor nuclei and in the spinal cord. 2. All groups can be recognized in premetamorphic larvae as well as in juveniles. 3. The number of projection neurons increase comparably in the vestibulo-ocular and the vestibulo-spinal components of the vestibular complex. 4. Some of the projection neurons change their terminal fields in the rostral eye motor nucleus from pre-metamorphic unilateral terminal fields in the larva to bilateral termination after the period of eye migration in juveniles.

We conclude that the vestibular complex is basically similar in flounders and other teleosts. The reorganization of terminal fields in vestibulo-ocular pathway during metamorphosis may be part of the plasticity required to compensate for the eye migration.

586.15
MAP KINASE KINASE (MEK-1) IS ENRICHED IN RADIAL CELL PROCESSES IN ZEBRA FINCH BRAIN. J.M. George*, H. Jin, W.S. Woods, and D.F. Clayton. Dept. of Cell and Structural Biol., Univ. of Illinois, Urbana, IL 61801.

A differential screening strategy previously led to the identification of cDNA clones representing RNAs enriched in parts of the songbird telencephalon (Mol. Br. Res. 12:323, 1992). The sequence of one of these clones (HAT-5) predicts a protein 95% identical to the recently cloned mammalian MEK-1 protein (Crews et al., Science 258:478, 1992), which functions as an activator of MAP Kinase/ERK-1 and -2, as part of the signal transduction pathway for various growth factors. By Northern analysis, the HAT-5 RNA is most abundant in the songbird neocortex (Zebra finch, canary), but is noticeably enriched in the brain. Expression in brain is minimal in newly hatched finches, moderate at 7 days of age, and much higher still in adults. An antibody raised against residues 38-50 of the MEK-1 peptide reacts with a band of ~48 kD on immunoblots of brain extracts. By immunocytochemistry, the antibody stains cellular processes that appear to arise from the vestibular lining, the tegmental plate at the tips of the ventricles. In Nissl-counterstained sections, the processes are occasionally associated with small elongated cells. Processes with these features have previously been identified as elements of radial cells, which serve both as neuronal precursors and as substrates for migration of young neuroblasts in the adult avian brain. The enrichment of MEK-1 in radial cell processes suggests the radial cells may respond to signals generated deep within brain tissue, far from their soma in the vestibular lining. [Supported by NIH: NS 25742]

586.14
FUNCTIONAL DESENSITIZATION OF NMDA RECEPTORS IN AN ANIMAL MODEL OF CHRONIC UP-REGULATION OF GLUTAMATE RELEASE. P. Marinii, M. Di Luca, A. Caputo, L. Pastore, M. Camgo, G. Bonamico, M. Raineri, J. Perez-Portela and F. Cuello. Institute of Pharmacological Sciences, University of Milano, 20133 Milano and Institute of Pharmacology and Pharmacognosy, University of Genova, 16148 Genova, Italy.

Rat exposed in utero to methylazoxymethanol (MAM) at embryonic day 15 show a profound disorganization in the CA region of the hippocampus. These animals show cognitive deficits and impairments in LTP induction (Ramakers et al., 1993). At a molecular level, in the presynaptic compartment increased phosphorylation of B-SUGAR-43 is present with a parallel and persistent increase in the membrane-associated PKC. As a consequence, increased basal glutamate release (352±23 pmol/log protein control; 575±50 pmol of protein-MAM-treated) has been observed in hippocampal synaptosomes of MAM-treated rats, whereas basal release of GABA is unaltered.

The functional state of NMDA receptors was evaluated in hippocampal plasma membranes by 3H-MK801 binding under nonequilibrium conditions, with a mathematical model allowing for the study of simultaneous modulation of the receptor by two agonists, Glycine and Glutamate, according to Marvizon and Baudy, 1993. The data show that in MAM-treated rats the allotropic interaction between Glycine and Glutamate sites shows a 10 fold difference with respect to controls, without changes in the number of binding sites as measured by [3H]-CGP38653 binding.

It is known that phosphorylation processes can influence the function of NMDA receptor. In these animals in vivo PKC dependent phosphorylation of the post-synaptic substrate neurogranin is markedly altered, suggesting that alterations of plasticity mechanisms involve pre- and post-synaptic parameters. Marvizon J.C. and Baudy M. Analyt.Biomed. 213-31, 1993. Ramakers G.M.J. et al., Neurosci. 54:49-60, 1993.

586.16

Previous studies of differential gene expression in songbird brain led to the identification of a brain-specific novel protein, HAT-3 (George et al., 1993). This sequence predicts a 143 amino acid protein which contains a repeating amphiphatic motif of 11 amino acids. Antibodies raised against the predicted C-terminus detected a single band (~17 kD) on immunoblots of both songbird and rat brain synaptosomal extracts, and stained zebra finch brain sections in a pattern consistent with synaptic localization.

With the ultimate goal of analyzing the function of the novel protein, we have used these antibodies to stain cultured hippocampal neurons from rat brain. Before synaptic contacts are present (e.g. 1-3 days in vitro [DIV]), HAT-3 is present in the cell body but appears diffuse and in low levels in processes. The antibodies yield a punctate staining pattern in more mature neurons (e.g. 7 DIV or older) that have established synaptic contacts. In double staining experiments, the protein is colocalized at the light microscopic level with the well-characterized synaptic vesicle protein synapsin I. Double staining with MAP2 revealed that HAT-3 puncta were concentrated around the soma and along dendrites. These results suggest that, while initially diffuse, in later stages of development HAT-3 protein becomes concentrated at regions of synaptic contact, and is localized presynaptically. Supported by NS17112 (GB), NS07189 (GW), HD07333 (AS), NS05742 (DC).

Closed head injury has been associated with cognitive and behavioral deficits in humans. In this study, we examined the effects of such injury on the circadian system. Wistar rats were implanted with biotelemetry transmitters, and circadian temperature and activity rhythms were recorded under constant darkness (DD) conditions for 10 days. Pentobarbital-anesthetized rats were then mounted in a stereotaxic apparatus and injected using a 25-gm weight drop device. Behavioral and core temperature rhythms were altered by closed head injury. Both cold light and pentobarbital anesthesia reduced the amplitude of these rhythms. These results suggest that closed head injury alters circadian rhythmicity. Colony environments and potential head injury might be secondary to the effects of such damage on the circadian system.
LOCAL SYNAPTIC CIRCUITS IN THE RAT SUPRACHIASMATIC NUCLEUS.

Previous anatomical studies have indicated that a large fraction of neurons and presynaptic terminals in the suprachiasmatic nucleus (SCN) contain GABA. Furthermore, electrical stimulation of regions outside the SCN has been found to produce GABAergic synaptic potentials in SCN neurons, but it is unclear whether such evoked potentials arise from the stimulation of GABAergic neurons within the SCN, or of fibers originating in other areas. To test whether SCN neurons from local lateral geniculate nucleus (LGN) neurons, we applied brief pulses of glutamate (10 mM, 0.2 s) to the SCN during whole-cell voltage-clamp recording in thin (150 μm) hypothalamic slices. Such glutamate pulses would be expected to stimulate projection fibers rather than fibers of passage. Whole-cell voltage-clamp recordings in SCN from 12 to 18 day-old Lewis rats revealed spontaneous outward currents in all neurons (n = 33), with amplitudes ranging from 20 to 200 pA at holding potentials near 0 mV, and a frequency of occurrence ranging from 0.03 to 10 Hz. Bicuculline (10 μM) blocked these events in 9 of 9 neurons, indicating that they were GABA-mediated inhibitory postsynaptic currents (IPSCs). Pressure ejection of glutamate-containing bath solution onto the SCN resulted in clear increases in the rate of IPSCs in 6 of 18 neurons. Spontaneous inward currents, which were presumably presynaptic postsynaptic currents (PPSCs), were seen in fewer neurons (n = 11 of 15), possibly due to their lower rate of occurrence (about 0.1 Hz, on average). Glutamate microinjection did not evoked IPSCs reliably in any of 12 cells. These results suggest local synaptic circuits within the SCN exist, and are predominantly inhibitory.

Supported by a grant from the AFOSR.

LOCALIZATION OF PRETECTAL NEURONS IN THE RAT WITH EXTENSIVE PROJECTIONS TO THE SUPRACHIASMATIC NUCLEUS (SCN) AND INTERGENICULATE LEAFLET (IGL).

A characteristic anatomical feature of the two most important components of the mammalian circadian timing system: the SCN and the IGL, is that they are bilaterally innervated from the retina. Parts of the olfactory and posterior pretectal nuclei have been shown to be bilaterally innervated from the retina as well, and we therefore aimed to explore whether these two nuclei were anatomically related to the circadian system. We examined retrograde neuronal labeling with Fluoro-Gold, Phaseolus vulgaris-leucoagglutinin (PVAL), and biocytin. Injections were given in the IGL and SCN of adult male rats. Only if PVAL was injected in the medial part of the pretectum it was possible to get the IGL and pretectal nuclei labeled. PVAL and biocytin filled fibers were observed to course laterally and rostrally into the optic tract, and, within the optic tract and chiasm, the biocytin filled fibers penetrate medially and descend rostrally into the SCN. Along this pathway, the projection gave rise to a large number of branches, across the superior, middle, and inferior IGL, and the entire IGL and the SCN. The presence of labeled neurons scattered in both the posterior and olfactory pretectal nuclei was observed in both experiments. Most neurons were found in the medial part of the posterior pretectal nucleus and in the dorsal-medial part of the olfactory pretectal nucleus ipsilateral to the injection, confirming the anterograde tracing studies. These neocellular tracts tracing reveal the presence of a novel pathway linking the pretectum, the SCN and the IGL, indicating that the projecting pretectal neurons and their efferent projections are potentially involved in phase-shifting mechanisms.

OVOGENY OF THE MOTOR CIRCUIT SYSTEM IN CRAYFISH.
J. Hernández-Falcón A. de la O-Martínez and P. Fuentes-Pardo, Depto. Fisiología, Facultad de Medicina, UNAM, AP 70-250, México, 04510, D. F., MEXICO.

The ontogeny of motor circuits of the crayfish (Procambarus clarkii) was studied using specimens kept under controlled conditions of light and temperature (17°C). The motor activity of animals aged between 10 to 100 days after hatching was recorded. During 10 days the movements of unrestrained crayfish were monitored by means of a video camera coupled to a digital recorder. The recordings were obtained under free-running in darkness (DD), free-running in light (LD) and LL 12:12 (LD). From each recording in order to obtain a measure of periodogram parameters (amplitude, : ratio and night/day ratio) were measured; was calculated by means of a computer program. From each periodogram the mean of 10 days the mean of 6 periods was calculated by means of a computer program. The mean of the mean of 6 periods was 25.9 h in both LL and LD. The lowest mean of the mean of 6 periods was 22 h in LL, 23.9 h in DD, and 20 h in LD. The crayfish in LD was more irregular than the locomotor patterns. However, the ability to be synchronized by LD photoperiods is better in the younger than in the older crayfish. The results indicate that the circadian organization appears very early in the development. They suggest also a progressive increment in the number, kind, and position of division of the oscillator during the ontogenetic development.

Supported by DGAPA IN 202292 grant.

DIFFERENTIAL INNERVATION OF THE HAMSTER SUPRACHIASMATIC NUCLEUS (SCN) AND INTERGENICULATE LEAFLET (IGL) BY RAPHÉ NUCLEI. E.L. Meyer* and L.P. Morin, Dept. Psychiatry and Neurobiology, Stony Brook University, NY 11794.

The SCN and IGL, components of the circadian system, receive serotonergic (5-HT) innervation from the dorsal (DR) or median (MR) midbrain raphe. In the rat, the data are inconsistent as to which nuclei projects to SCN or IGL. To study DR and MR pathways to the hamster circadian system we have not performed. Retrograde (Fluoro-Gold, cholera toxin, Fast Blue) and anterograde (Phaseolus vulgaris leucoagglutinin; PHA-L) tracing techniques were used to show raphe neurons and their projections. Retrograde tracers were injected into the SCN or IGL of male golden hamsters and locations of labeled cells in DR and MR were shown. PHA-L was also injected into the DR or MR and the projections to the SCN and IGL were mapped.

The autoradiograms show the DR, but not projections to the IGL. Retrograde labeling placed in the SCN labels cells in the MR, but not DR. In contrast, placement of PHA-L in either the DR or MR labels fibers in the IGL. Retrograde analysis is in progress. The results are consistent with data (unpublished) showing that, but not DR, lesions destroy 5-HT projections to the SCN, DR, but not MR, lesion show 5-HT fibers to the IGL. The anterograde labelled MR fibras to the IGL may not contain 5-HT. This issue is being evaluated. Supported by NSF21168.

THE SUPRACHIASMATIC NUCLEUS AND INTERGENICULATE LEAFLET OF ARVICACHUS NILOTICUS, A DIURNAL MURID RODENT.
L. Smale*, Dept. Psychology, Michigan State University, East Lansing, MI, 48824.

Little is currently known about how the neural substrates controlling circadian rhythms differ in diurnal compared to nocturnal species. Numerous studies have been carried out on rodent models. Arvicanthus niloticus, a murid rodent recently imported from Kenya, exhibits a diurnal pattern of precisely timed wheel-running activity and may represent an ideal diurnal mammal for studies of the neural control of circadian rhythms. Arvicanthus niloticus is a murid rodent that has been adapted to nocturnal activity, and has been used for many years as a model of circadian rhythms. The use of this species as a model of circadian rhythms has not been widely utilized. In this study, we will use immunohistochemistry to examine the distribution of peptides in the SCN and IGL of 8 adult A. niloticus (2 females and 6 males); in this initial study animals were not pre-treated with colchicine. In A. niloticus, as in other mammals, distinctly different subdivisions of the SCN contained VIP and VP immunoreactive (IR) cell bodies. Within the SCN, we did not observe cell bodies containing npy, naitkalin or substance-P. Immunoreactive NPY-IR, ENK-IR and VIP-IR fibers were present within the IGL. The IGL contained a substantial number of VIP-IR cells, as well as SP-IR and ENK-IR terminals. Overall, the IGL and SCN of A. niloticus resembled, in many respects, those previously described in rats.

EFFECT OF SKELTON PHOTOTOPÆRES UPON THE CARDIAC MOTOR ACTIVITY RHYTHM DURING ONTOGENY IN CRAYFISH.

During the development of the crayfish Procambarus clarkii, the synchronization of the circadian activity rhythm (CAR) to complete photoperiods (CP) is not clear (1) probably due to a masking effect of light. We tried to rule out this effect, studying the influence of the syetehetical skeleton photoperiods (SSP) upon the CAR at different stages of development, Procambarus clarkii juvenile instars between 10 up to 150 days after hatching were individually housed in activity recording cages under constant darkness and temperature conditions. Each crayfish was free running during 15 days. Afterwards, it was changed to the following CP: 12:12, 8:16 and 20:4. After 15 days of SSP stimulation they were left free running again. The results suggest that only animals older up to 60 days are able to synchronize to SSP adjusting its maximal phase to the longest scotophase.


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588.1

The aim of the present work was to study the effects of lesions in the intergeniculate leaflet (IGL) or the deep ponte / lamina intercalaris region (DP) on the diurnal profile of N-acetylserotonin (NAS) and on the nocturnal pineal inhibition process induced by acute light exposure. Male adult albino rats (n=2/12, 12h:12l-dark cycle, lights on at 0600h), intact or previously lesioned, were killed along the 24 hours or during the night immediately after 1 or 15 minutes of exposure to 500 lux white lights. The pineal glands were collected and frozen (-70°C) until NAS was assayed by HPLC-ED. The 24-hour experiment shows that there is no phase shifting on the diurnal NAS curve of groups of rats with large bilateral IGL lesion compared to the controls. On the other hand there is a significant reduction in the amplitude of pineal NAS content observed in every nocturnal point of the curve (p<0.05). The pineal glands of IGL-lesioned rats do not respond to bright (1 min.) retinal photostimulation keeping its NAS content equal to the lesioned dark killed rats (p<0.05). On the other hand, 15 min. of photostimulation bring the pineal NAS content of the IGL group to nearly zero evenly to the control animals. In experiments done at 2400 h, DP lesion does not modify the content of NAS in the pineal gland of rats killed in the dark. However, the pineal photo-inhibition process induced by 1 min, of light exposure is impaired since the lesioned group has its NAS content reduced much less than the intact animals. These results suggest that: 1) the nocturnal photostimulation input of the pineal gland is partially dependent on central and direct neural projections, probably from the IGL region; 2) The IGL exerts a tonic excitatory control on the nocturnal pineal NAS production, probably via its indirect projections controlling the hypothalamo-spinal cord-sympathetic pineal system.

588.2
INTRACELLULAR CA\textsuperscript{2+} DYNAMICS IN DISCONNECTED CELLS OF THE CHICK PINEAL GLAND. T. D’Souza & S. F. Dryer*. Program in Neuroscience, Florida State University, Tallahassee, FL 32306.

The regulation of intracellular free Ca\textsuperscript{2+} concentration was examined in disconnected chick pineal cells using the fura-2 technique. Approximately 10% of cells examined exhibited spontaneous Ca\textsuperscript{2+} oscillations. Membrane depolarization evoked large increases in intracellular calcium which were dependent upon external Ca\textsuperscript{2+} ions. Application of thapsigargin (2 \muM) evoked increases in intracellular free Ca\textsuperscript{2+} in the absence of external Ca\textsuperscript{2+}, indicating the presence of internal stores. Application of 100 \muM caffeine (100 nM), forskolin (200 nM) evoked sustained increases in intracellular Ca\textsuperscript{2+}. The responses to forskolin could be observed in Ca\textsuperscript{2+} free external saline suggesting mobilization of internal stores. These results suggest new mechanisms for the regulation of melatonin synthesis and secretion, and possible sites of action for the intrinsic circadian oscillator. Supported by AFOSR F49620.

588.3

ML-1 receptor binding sites have been localized within a number of circadian and visual areas of the mammalian and avian CNS. We have localized and characterized [Z2(-2)-I]-iodomelatonin ([Z2(-2)-I]ME) binding sites in guinea-pig brain. Specific [Z2(-2)-I]MEEL binding, defined using melatonin (1µM), was identified in coronal sections of guinea-pig brain by receptor autoradiography at the level of the arcuate, suprachiasmatic and lateral geniculate nucleus, superior colliculus (SC) and nucleus accumbens. In SC and hypothalamic membranes, high-affinity, specific, saturable and reversible binding was observed. In the SC, [Z2(-2)-I]MEEL is bound to a single population of high-affinity binding sites (Kd 14.16±6.13 pm, Bmax 662±46fmol/g tissue), while in hypothalamic both high and low affinity sites were identified (Kd 33.5±6.5 pm, Bmax 19.1±10.7fmol/g tissue; Kd 6.6±2.8 pm, Bmax 153±47fmol/g tissue), which appear to represent two affinity states of the ML-1 receptor. In hypothalamic, melatonin agonists inhibited binding with a rank order of potency (5-CIT: [Z2(-2)-iodomelatonin]: 1-iodo-C-tryptophan: 1-iodo-Cl-tryptophan: 0.25µM) > NQZ-C-7-(methylthio)-tryptophan which (1.8±0.2 mg/ml > 6-chloromelatonin (1.1±0.6 mg/ml) > N-acetyl-1-iodomelatonin (1.4±0.7 mg/ml) > 5-HT (>100µM), 5-methoxytryptamine-N-acetyltryptamine (0.2 mg/ml), which has higher affinity for N-acetylserotonin-preferring (ML-2) receptors (Dubocovich et al., JUPHR, 1994), was only weakly active (<1µM). The rank order of potencies in SC membranes was identical (correlation coefficient 0.998).

In conclusion, [Z2(-2)-I]MEEL binding sites, with the characteristics of ML-1 receptors, were localized in guinea-pig SC and hypothalamic areas, in which this receptor may regulate visual and circadian information, respectively.

588.4
NON G-PROTEIN COUPLED HIGH AFFINITY MELATONIN RECEPTORS ARE PRESENT IN THE SHEEP BRAIN. Barret, P. L., Lawson, W., MacLean, A., Hazledin, D., Williams, L. M. and Morgan P.J. Rowell Research Institute Buckholt, N.T, Australia, Commonwealth Scientific and Industrial Research Organization (CSIRO) / NCI. All Melatonin receptors have been localised to both neuronal and non-neuronal sites by in vitro autoradiography using the radioligand [3H]-iodomelatonin. In general these receptors are characterised as members of the G-protein class, as they are membrane-bound, of pico-molar affinity which is modulated by guanine nucleotides. In human cerebral and pinealocytes, melatonin receptors to inhibit forskolin-stimulated adenylyl cyclase through a coupling to two independent inhibitory G-proteins. Recent evidence suggests that unique G-like G-proteins, which is both a cloned cholinergic subtype and recognised by olsubt4 C-terminally directed antibodies mediate this response. In contrast sheep neuronal tissue has markedly different characteristics. Homogenate binding assays reveal that the hippocampal binding site has high affinity (Kd = 56 pm), yet binding is not affected by GTPyS, choloria or pertussis toxins, each of which modulate the affinity of the PT melatonin receptor. Native polyacylamide gel electrophoresis resides the PT receptor as a complex which migrates with a molecular mass of ~515kDa. Under the same conditions the hippocampal receptor migrates with a molecular mass of ~360kDa. This assay also provides a visual demonstration of the lack of GTPyS sensitivity of the hippocampal receptor. With this technique we also show that the melatonin receptor present in the cerebral cortex and pre-optic area has a similar mass to the hippocampal receptor and are not affected by GTPyS. This suggests that these receptors are not G-protein coupled and therefore belong to different class of receptor.

This work was funded by SOAFA

588.5

Sleep-inducing properties of low doses of melatonin examined in young healthy volunteers. The hormone (0.3 or 1.0 mg, p.o.) or placebo was administered at 1800 or 2000 h; the lower dose elevated serum melatonin to concentrations comparable to those normally occurring nocturnally in adults (80-120 pg/ml). Effects of the treatment on performance and mood were monitored all day prior to evening treatment and until 0900 the next morning. The volunteers' subjective evaluation of the hypnotic effect showed that they could distinguish between the treated and placebo group, especially after treatment at 2000 h. Beginning 2 hours after treatment, sleep latency was measured by a switch release or by a polysomnographic system. Either dose given at either time point decreased both sleep latency and the sensitive index of melatonin's effect, sleep stage 2 latency. These observations indicate that raising blood melatonin levels to those normally occurring nocturnally promotes sleep onset in humans.

The pineal melatonin rhythm in a variety of rodents is suppressed by exposure to 60 Hz magnetic fields (MF) and may alter the clock mechanism involved in the photoperiodic control of reproduction (U Cell Biol 31: 394, 1993). To determine if MF disrupts photoperiodic regulation of sexual maturation, males in long (14L:2D) or short (12L:12D) days were exposed each day to a 1 Gauss MF (15 min, beginning 2 h before lights on) for 2 days, with 18 days of development. At 25 days of age, the MF exposure continued for 4 days. Males were housed singly and maintained on a 10-10 hr light-dark schedule. The numbers of the hamsters were measured in circadian rhythm, and the melatonin activity was determined by radioimmunoassay.

588.8 MELATONIN DURATION AND REPRODUCTIVE RESPONSES IN MALE SYRIAN HAMSTERS. J.B. Powesky*, A.E. Jetton and R.A. Mascola, Departments of Psychology and Biology, Univ. of Massachusetts, Amherst, MA 01003-7710.

Syrian hamsters are long day breeders, exposure to short days causes inhibition of the reproductive system. This response requires the nightly secretion of melatonin which serves as a hormonal indicator of night length. Traditional views suggested that day length shorter than 12.5 hr were equally effective in generating reproductive inhibition, independent of the actual day length used and its associated melatonin duration. In the experiments presented here we determined if variations in melatonin duration or photoperiod history would affect the rate of reproductive inhibition following melatonin treatment. Forty-five male Syrian hamsters were exposed to long days (16L:8D) for two months and then assigned to one of four groups; three remained in 16L and were pseudoexcised (PDNX); the fourth was shifted to a short day (7L:17D) and was sham pseudoexcised (SH-PDNX). These PDNX groups received nightly infusions for nine weeks containing either vehicle (VEH) or melatonin (MEL, 50 mg/hr) for 8.5 hr or 12.0 hr. The SH-PDNX group was not infused. At three week intervals, testis weight were measured and blood withdrawn for assays of follicle stimulating hormone (FSH) and prolactin (PRL). Both MEL durations caused significant testicular regression but this occurred more rapidly with the longer MEL duration (p<0.05). In a second experiment, MEL duration was held constant but groups were exposed to differing long days prior to MEL treatments. Forty-eight males were divided into two groups - one exposed to 16L:4D, the other to 14L:10D for six weeks. All hamsters were then PDNX and switched to 16L:8D. Within each condition, one-half the males were infused for 9.5 hrs each night for nine weeks with either MEL or VEH; their reproductive condition was assessed as in the first experiment. MEL infusions caused significant reproductive inhibition (p<0.05) but this was only moderately influenced by photoperiod conditions prior to MEL treatment. Results derived from serum assays of FSH and PRL will be reported. Supported by HD30372, HD70673 and MH44132.

588.9 MELATONIN INDUCES OUTWARD CURRENTS IN A SUBPOPULATION OF RAT SUPRACHIASMATIC NUCLEUS (SCN) NEURONS. Z. C. Jiang and C.N. Allen, Center for Research on Occupational and Environmental Toxicology and Dept. of Physiology, Oregon Health Sciences University, Portland, OR 97201.

Several lines of evidence support a role for melatonin in entrainment of circadian rhythms via direct actions on SCN neurons. However, the cellular mechanisms of melatonin's actions remain largely unknown. We used whole-cell recording techniques and brain slices to examine the membrane responses induced by melatonin. Young adult male rats were housed on a 12:12 h light-dark cycle and their locomotor activity and body temperature monitored for 2.4 weeks before the experiments. Full entrainment was observed in the light-dark schedule was always achieved in less than a week. Animals were killed 3.5 h (CT3-5) after lights off and recordings were made during the next 1-0 h (CT3-5). Melatonin (0.1-33 nM) induced an outward current (3.40 pA, at -60 mV), in 4 of 20, 8 of 27 and 5 of 18 cells tested between CT6-CT9, CT9-CT12 and CT12-CT15, respectively. The current was associated with an increase in membrane conductance. The response concentration-dependent with a threshold of about 100 nM and a maximum effect at 30-300 nM. The melatonin-activated currents were blocked by 1 mM Ba2+, partially blocked by 3 mM Cs, but not affected by TTX and/or a combination of CNQX and APV. The current amplitude was reduced and, in a few cases, reversed by a hyperpolarization over -100 mV. These data suggest that melatonin activates a potassium current thus causes inhibition in a subpopulation of SCN neurons. Study supported by grant AG10794.


Department of Animal Sciences, University of Nebraska, Lincoln, NE 68588.

Ovine parabasilis (oPT) cells containing melatonin receptors, and parabasal (oP) cells lacking such binding sites, were studied for the intracellular transduction of melatonin signal. oPT cells, which are involved in the level of CREB activation, measured as P-CREB immunoreactivity (P-CREBir). Gel shifts identified two specific CRE oligonucleotide-protein complexes in oPT nuclear extracts. These were displaced by control and different CRE oligonucleotides and both bands were supershifted using anti-CREB and anti-P-CREB antisera. Immunocytochemistry results were quantitated using digitized image-analysis to measure oPT nuclei containing CREB or P-CREBir. The CREB was increased in the nuclear optical density. Increased PCREBir in response to forskolin stimulation was time and dose dependent. This change in staining was not detectable using the anti-CREB antibody, hence increased PCREBir is due to rapid phosphorylation rather than de novo CREB synthesis. The time course for forskolin stimulated P-CREBir was consistent with nuclear translation of protein kinase A. In oPT but not oP cells, CREB phosphorylation was inhibited by melatonin in a time and dose dependent manner, with the dose also being dependent on the forskolin concentration used. The IC50 for melatonin was around 10nM. Melatonin is therefore thought to regulate expression of cAMP-responsive gene expression through exerting an inhibitory effect, via the cAMP/PAK pathway, on the level of activation of the transcription factor CREB. Funded by SAFRD and Welcome trust.


Pineal calcifications increase with advancing age in man and in many other mammals. Melatonin biosynthesis decreases with advancing age, and has several calcium dependent steps, suggests the possibility of pineal cell dysfunction related to the aging process may accelerate biomineralization in the pineal gland. Pineals from a range of aged men (14, 47, 62 and 82 years) were metallographically embedded, polished, and studied by scanning EM and transmission EM for age related differences. The data show: 1) Concentratically aligned crescent shaped lamellae increase in number and decrease in width, with advancing age. This suggests remodelling age. 2) There is an increase in the calcium: phosphorus ratio (1.27-1.41 in 14 to 62 year age), with advancing age (1.49-1.62 in 82 years) in all lamellae. CIP lamellar increases may parallel cell dysfunction and dysfunction. 3) The architecture of the deposits show an age progression from a) round smooth-stage, through a round beaded stage and into acrocelli (i.e. arteriol) stage. This suggests mineralization and growth by method other than simple apposition. These results suggest a biomimeralization followed by a remodeling that changes throughout the organism's life span and may relate to the decline of melatonin biosynthesis.


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Aerobic exercise training (ET) modifies the behavioral responses to acute stress. The effects of ET on the response to stress are less clear. The purpose of this study was to examine the effectiveness of ET in attenuating the behavioral and hormonal responses to a stressor, namely, 20 min of restraint stress (CUS). 16 Flinders Sensitive Line (FSL) and 16 Flinders Resistant Line (FRL) rats were used in this investigation due to their respective sensitivity and resistance and subjected to 3 weeks of ET (25 m.min⁻¹ and 7% grade, 30 min per day) or remained sedentary (SED). Animals were then subjected to 1 wk of CUS. Open field mobility (OFM) and sucrose preference (SPC) were evaluated prior to and after CUS. After CUS, blood was collected for measurement of plasma corticosterone (CORT). Following CUS the number of squares entered during OFM was reduced (p<0.05) in sedentary animals (both FSL and FRL); however, the number of squares entered during OFM was unchanged in FSL-ET and FRL-ET. The number of rears during OFM was reduced (p<0.05) in all animals following CUS. Following CUS, SC consumption was reduced (p<0.05) in all groups except FRL-ET. Plasma CORT was greater in FSL-SED than FRL-ET following CUS. In comparison, plasma CORT values in FSL-SED and FSL-ET were similar to normal resting values. These results indicate that ET is effective in attenuating some, but not all, of the behavioral responses to CUS. Furthermore, FSL animals did not benefit as much from ET as FRL animals. Resistance to the behavioral effects of stress by ET was associated with lower CORT levels in FRL animals.

EFFECTS OF MILD INTERMITTENT TAIL SHOCK ON COLONIC MOTOR ACTIVITY. N.S. Morrow* and T. Garrick. Center for Ultra Research and Education, Dept. of Veteran Affairs, Palo Alto Medical Center, Los Altos, CA 94324.

Changes in the motor activity during tail shock were examined in rats chronically implanted with 2 force transducers into the colon. Baseline colonic contractions were monitored in home cages for 3 days. Meal-stimulated changes in colonic activity were then examined in 2 testing periods. Following a scheduled feeding trial, rats were randomly divided into 3 groups (n=6 each) for testing. All rats were loosely restrained in a Plexiglas tube, and 2 electrodes taped to the tail. One group of rats was administered unpredictable, intermittent tail shocks, 1.5 sec VI-1, 4 shocks/5 min. A second group was exposed to the tail shock chamber but was not shocked. The third group of rats received tail shocks for 1 h and then 24 h later were re-exposed to the tail shock chamber but were not shocked. Fecal output was recorded throughout the experiment. The number of feces evacuated decreased significantly (p<0.05) when compared to basal values. Tail shock increased the number of feces evacuated (p<0.05) and affected fecal consistency in a variable manner. Exposure to the shock chamber, alone, did not significantly alter the colonic contractility pattern or fecal output from basal values. These results indicate that alterations in colonic motor activity can be induced by exposure to environmental stressors and may be a contributing factor in stress-induced bowel dysfunction.


Prenatal stress and neonatal handling are known to have long-lasting effects on emotionality. The hippocampus mediates, at least part of, these effects. In some cases of stress exposure, the hippocampus effects are beneficial, whereas in some cases more detrimental effects may be seen. In this study we investigated the effect of prenatal stress and of neonatal handling on spatial ability and learning, behaviors regulated by the hippocampus. From GD 14 to 21 pups were exposed to shock, or no shock, for 45 min 3 times/day. From PN 1-10, half of the pups were handled daily by placing them in a cage at 37°C for 3 min. At approximately 23 and 63 days rats were tested in a Morris Water Maze (MWM). Prenatal stress and handling on a MWM, nor did handling. A sex difference in latency to find the platform, was observed in adult animals, males performing better than females. These results were confirmed in prepubertal animals. No age difference was also observed, with older animals learning to reach the platform twice as fast as prepubertal animals. Our previous results show that the prenatal stress treatment was effective in reducing the number of squares entered during a 20 min of restraint stress (CUS). These animals were then subjected to a social defeat experience for 5 days with submissive behavior and ultrasound vocalizations monitored. Changes in the amplitudes of cardiac and ultrasonic rhythms following social stress and light shift and entrainment effects following drinking were observed.
589.7
THE IMMEDIATE EFFECTS OF PHYSICAL EXERCISE ON PASSIVE-AVOIDANCE MEMORY AND ANXIETY IN RATS. J.E. BRVAT, Tracey Smith, Leslie R. Hicks and Michael J. Lewis. Neurobehavior Lab, Dept of Psych, Howard University, Washington, D.C. 20059

Participation in physical exercise is currently fashionable. Although many people report psychological benefits following exercise, the present research was designed to investigate the effect of physical exercise on passive-avoidance behavior and anxiety in rats. The subjects were 32 female, 250 g Sprague-Dawley rats divided into four groups of eight. All rats were given 20 min daily training sessions of an eight-trial, two-choice, passive-avoidance task. Following training, behavior was assessed in the active-avoidance and forced-choice tasks. Group A was exposed to a 10 day running regimen, Group B served as a control group. Group C received 10 days of running training, followed by a 10 day running regimen, Group D served as a control group. The exercise program was designed to increase VO2max by 30%. Passive-avoidance behavior was assessed by the latency to enter zone A, and forced-choice behavior by the latency to respond to zone A. The results were analyzed by 2 x 2 x 2 x 2 ANOVA. The analysis revealed significant main effects of running and non-running on active-avoidance performance, and a significant interaction of training and non-training on forced-choice responses. It was concluded that exercise training had positive effects on anxiety and passive-avoidance memory in rats. This finding is consistent with previous studies. Further studies are needed to determine the underlying mechanism of this effect.

589.8
EFFECTS OF INDUCED POSITIVE AND NEGATIVE STRESSORS ON IMMUNE FUNCTIONING IN CHRONIC DEPRESSION AND CONTROLS. A. V. Raymond, J. Griffiths, S. Zalcman & H. Anisman. Royal Ottawa Hospital, Ottawa, Ontario, Canada KIZ.TK4.

Stressful events encountered by humans have been reported to influence immune functioning. Depending on severity, stressors may influence natural killer (NK) cytotoxicity, circulating lymphocyte subsets (e.g., NK cells), as well as cell proliferation in response to mitogens. In the present investigation, a brief mild laboratory stressor (cognitive challenge) increased NK cell numbers in controls and in dysthymic, low grade depressive patients. The extent of the increase was directly related to plasma norepinephrine levels, and may reflect effects on cell trafficking. Likewise, a more meaningful stressor (having subjects complete the day-to-day (CS) task, leading to subsequent stressors) resulted in an increase of NK cell numbers in both dysthymic and control subjects. Cell proliferation in response to mitogens was reduced in patients who experienced these stressors.

These effects were less marked when patients discussed uplifts, or in control subjects who discussed their hassles. Finally, control subjects who had undergone a stressor perceived as being more severe (final academic examination) exhibited NK cell alterations, as well as variations of several other lymphocyte subsets (e.g., CD4, CD3 and CD-19). The data discussed in terms of the relationship between stress and depression and the impact of stressors on immune functioning.

589.9
EXAGGERATED ACOUSTIC STARTLE IN GULF WAR VETERANS WITH PTSD. C. A. Morgan III, C. Griffiths, S. Southwick, and D. S. Charney. Department of Psychiatry, Yale University School of Medicine, West Haven VAMC, West Haven CT 06516.

Exaggerated startle is reputed to be one of the cardinal symptoms of Post Traumatic Stress Disorder (PTSD). Objective studies have given conflicting results as to whether or not startle is increased in PTSD. However these studies were conducted on subjects with chronic PTSD (Vietnam and Israeli combat veterans). The present study investigated the acoustically startle response of eight Gulf War veterans with acute PTSD and 15 healthy age matched controls. The eyelid component of the startle reflex was measured in response to six blocks of pulses (40 ms) white noise bursts of varying intensities (90, 96, 102, 108, 114, 120 dB). No war related or stressful cues were presented to subjects prior to or during testing. Startle amplitude was significantly greater and habituation significantly reduced in the PTSD subjects compared to controls. Because other studies in the literature, as well as our own laboratory, which have investigated the acoustically startle response in chronic PTSD, have failed to find exaggerated startle or reduced habituation of startle at baseline (i.e., absence of stress), it is likely that the present results reflect an acute elevation of startle in this group. The higher amplitude and decreased habituation of startle in the PTSD subjects may reflect a sensitization of the fear/alarms response created by the recent stress of combat trauma consistent with preclinical studies of shock sensitization and startle.

589.10

The purpose of the present study was to characterize the disruptive effects of chronic stress on the acquisition and terminal accuracy of a simple alternation task in rats. All rats lived in individual cages that were exposed to a 12:12 light/dark cycle. During the preshock period, two levers were available and any leverpress resulted in the delivery of a food pellet. Subsequently, rats in group A/E were trained to (Alvoid)/Eiscape signaled footshocks. After learning to escape, food was only available for alternating leverpresses. Alternation was also required in groups Y and C. In group Y, signals and shocks were yoked to those delivered to rats in group A/E; rats in group C were not shocked. This resulted in no difference between groups during acquisition, terminal accuracy (percent correct alternation) was significantly higher in group C than in the A/E or Y groups. This result is from a significantly higher mean number of correct presses (food pellets) for Group C than for either shock group. Since mean number of incorrect presses was significantly higher for group A/E than for group C or Y, the requirement to A/E footshock may have interfered with the accuracy of alternation.

589.11
DIFFERENTIAL EFFECTS OF ACUTE STRESS EXPOSURE ON RADIAL ARM MAZE PERFORMANCE. M.J. Stillman, R. Schug-Hale, A. Levy, H.E. Modrow, and H.B. Lieberman. Military Performance and Neuroendocrine Division, United States Army Research Institute of Environmental Medicine, Natick, MA 01760-5007, GEO-CENTERS, INC., Newton Centre, MA 02195, and HIB, Ness Ziona, ISRAEL.

This study examined radial arm maze (RAM) performance following exposure to two stress conditions and a normothemic-free moving control condition. Male Fischer 344 rats were trained on the win-shift RAM procedure for 7 days by which time they achieved asymptotic performance. The next day, rats were exposed to 15 min of restraint in either 37°C water (normothermic-restraint) or in 20°C water (cold-restraint). Rats were removed from restraint and were allowed 40 min in a dry cage prior to being tested in the RAM. Performance was measured using the following dependent variables: time per choice, the total number of choices, the percent error, and the number of correct out of the first eight choices (number correct). NOVA indicated significant effect of stress on number correct (p<.05) as well as the other variables. Performance decrements were observed in both stress conditions relative to the normothemic-free moving condition, with the normothemic-restrained rats displaying less impairment than the cold-restrained rats. From the data it was concluded that stress exposure, when compared to control conditions, had a significant effect on radial arm maze performance. Further studies are needed to determine the underlying mechanism of this effect.

589.12

In an attempt to characterize the pain tolerance response to naloxone administration following exposure to controllable stress, forty healthy male subjects were exposed to bursts of 95 dB noise while attempting to solve a visual-spatial task under either control (CS) or uncontrollable stress (UCS). No difference in pain tolerance was noted among groups during acquisition, terminal accuracy (percent correct alternation) was significantly higher in group C than in the A/E or Y groups. This result is from a significantly higher mean number of correct presses (food pellets) for Group C than for either shock group. Since mean number of incorrect presses was significantly higher for group A/E than for group C or Y, the requirement to A/E footshock may have interfered with the accuracy of alternation.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
PERSISTENT HIGH CORTISOL RESPONSES TO REPEATED PSYCHOLOGICAL STRESS IN A SUBGROUP OF HEALTHY MALES. C. Kirchbaum, J. Frittnier, A.A. Stone, I. Fedenor, J. Gaab, D. Lintz, N. Schonmer and D.H. Hellhammer*. Center for Psychology and Psychosomatics, University of Trier, 54286 Trier, Germany.

The present study tested the hypothesis that some subjects may not readily show habituation of adrenocortical stress responses to repeated psychological stress. 20 healthy males were exposed five times to the same, brief psychosocial stressor (public speaking and mental arithmetic in front of an audience) with one stress session per day. Salivary cortisol levels were assessed as an index of adrenocortical stress responses. For the total group, cortisol levels were significantly elevated on each of the five days. The mean response decreased from day 1 to day 2, however, no further attenuation could be observed on the remaining days. Consequently, we evaluated a subsample of subjects who showed completely different response kinetics. In the first group (n=13), termed "low responders", cortisol levels were elevated on day 1 only. Days 2-5 cortisol levels were unaltered. In contrast, subject in the second group ("high responders") displayed large increases to each of the five experimental treatments. This group had no significant response decrement from day 1 to days 2-4 and only a marginal response difference between day 1 and day 5. Discriminant analysis revealed that a combination of scores on a symptom checklist significantly discriminated between high and low responders. Using this discriminant function, all 20 subjects were correctly classified to the two groups. These results are discussed with a focus on the possible impact of adrenocortical stress response types on health and disease.

STRESS: NEUROTRANSMITTER STUDIES


To clarify information processing for novel stress in rat medial frontal cortex (mPFC), we developed newly system to monitor glutamate (GLU) and lactate with a high time-resolution (less than 1 min) by using in vivo brain microdialysis. The dialysate was mixed directly with an enzyme solution (containing GLU- and lactate-dehydrogenase and NAD+) in a Teflon-coated cartridge. The fluorescent of produced NADH in the mixture was measured continually by a fluorometer equipped with a flow cell. The basal level of mPFC GLU release was 60 pg/min and decreased 2-3pg/min a week. The basal lactate release was 90 pg/min and decreased 1-2 pg/min a week. These basal release rates were suppressed partially by the 2nd trial 1 hour later in a manner of habituation (GR; TP: 32.9 (+/-3.8%); LR: TP: 34.5; 7.1%; 100dB: 33.9-9.2%; IMB; 45.5-6.2% vs. the initial responses, all at least p<0.05 by paired t-test (n=4-5)), consistent with dishabituation to a novel stimulus or "bellowzer" habituation by several repetitions. GR and LR both showed the decreasing response by TP in septum. The mPFC GR release was appeared under reserpinein (0.5 mg/kg, sc) while these of LR were completely not. These results suggested that there are two different systems for stress processing compared in rat mPFC.


We obtained a chemical map of learned helplessness (LH) in the rat involving serotonin (5-HT), dopamine (DA) and GABA in medial prefrontal cortex, GABA and norepinephrine (NE) in hippocampus, and 5HT in septum. In hypothalamus, other investigators have reported decreased 5-HT uptake by sodium and K-stimulated release by tissue slices of LH rats, as well as decreased paroxentine and unchanged 5-HT-adrenergic receptor binding. We have now applied in vivo microdialysis to measuring DA, NE and 5-HT simultaneously in hypothalamus and caudate of conscious, freely moving rats in a LH paradigm. Rats received 100 trials of inescapable tailshock stress, and after testing, LH, microdialysis probes were implanted into either caudate or lateral hypothalamus. One day later, perfusion was maintained with Ringer's solution until a stable baseline was obtained, then switched to Ringer's with high K+. No differences were found in caudate in DA or 5-HT among any of the three experimental groups (LH, NH - stressed, non-stressed, or blank control). In hypothal,

*Adrenergic, no differences were seen for DA or 5-HT. NE in hypothalamus, significantly higher levels of basal NE were observed for LH rats, compared to NH and TC, while the NH rats had significantly higher K-stimulated NE release than LH or TC. For all the stressed rats, there was a significant positive correlation between shuttlebox escape latency and basal NE release. These data suggest that the caudate may not be involved in the in vivo biogenic amine neurotransmission of LH, and that in the lateral hypothalamus, NE mechanisms predominate in this animal model of depression.
590.5 EFFECT OF BETA-1 ADRENERGIC BLOCKADE ON c-FOS RESPONSE TO STRESS IN MOUSE BRAIN. Y. Zhang* and E.A. Stone. Dept. Psychiatry, New York University School of Medicine, New York, NY 10016

We have previously shown that beta-blocker-reversible c-fos expression can be used as a marker for postsynaptic noradrenergic activity at beta-receptors in the brain. The present study utilizes this method to determine in which regions of the brain beta-1 receptors are activated during stress. Male Swiss Webster mice were injected with the selective beta-1 blocker, beta-blocker-reversible c-fos immunohistochemistry using a fos-specific antibody by standard procedure. Noninjected-nonstressed and betaxolol-injected-stressed mice showed little c-fos immunoactivity in the forebrain. c-IMPO stress caused widespread c-fos expression in the forebrain and brainstem. Betaxolol treatment prior to stress significantly reduced c-fos in most forebrain structures but not in the brainstem. These results suggest that stress activates beta-1 receptors primarily throughout the forebrain. Supported in part by grants AFOSR F49620-92-J-0084, MH52655 and MH08618.

590.6 EFFECT OF PROPRANOLOL ON STRESS-INDUCED CHANGES IN PASSIVE AVOIDANCE AND OPEN FIELD EMERGENCE TESTS. I.S. Manavalan*, M. Najimi, E.A. Stone and D. Quartermain. Depts. Psychiatry & Neurology, New York University School of Medicine, New York, NY 10016

The present study examined the effect of propranolol on the ability of stress to elicit behavioral inhibition in Harlan Hsd:Nrd4 mice. Mice were given propranolol prior to immobilization or tube-restraint stress and then were tested for either passive avoidance performance or time to emerge into an open field. In contrast to previous findings by others, propranolol was found to markedly potentiate stress-induced increases in latency in these tests. These unexpected results were not due to peculiarities of the behavior measured, the stressors used or the dosage of the drug administered. As propranolol has blocking actions at beta adrenergic and serotoninergic receptors, the results raise the possibility that, under some conditions, the response of the noradrenergic and/or serotoninergic systems to stress may have anxiolytic or anti-stress effects. Supported in part by grants AFOSR F49620-92-J-0084, MH52655 and MH08618.


Recent experiments with rats in semi-naturalistic environments have indicated that chronic subordination stress impairs risk assessment, i.e., reduces the likelihood that the animal will engage in species typical behaviors to alter the location and source of possible danger. The present study was undertaken to determine if acute stress disrupts risk assessment in mice measured in terms of latency to emerge from a dark cage into a novel, potentially dangerous environment. Groups of male Swiss Webster mice (N=12) were either to immobilization, tube-restraint, unavoidable footshock or exposure to a dominant aggressive mouse. All stressors were administered to individual mice for a duration of 1 hr. Risk assessment behavior was examined 30 min following stress termination. Mice were placed in a small dark enclosed entry chamber which was attached to a large brightly lit open field which could be entered through a small door at the end of the chamber. Latency to emerge (max. 5 min) was determined. Immediately after, animals were observed in the open field for a 5 min period during which time locomotor activity was measured and rearing, grooming and defecation frequencies were recorded. Results showed: 1. Mean entry latency for all stress groups was significantly faster than that of a non-stressed control group. 2. All stressed groups showed significantly less locomotor activity and rearing and significantly more episodes of grooming than non-stressed mice during a 5 min period in the open field. These data indicate that acute stress can disrupt defensive behavior by impairing the animal's ability to respond to potentially threatening stimuli. Supported in part by grants AFOSR F49620-92-J-0084, MH52655 and MH08618.

590.8 THE ROLE OF NORADELINE IN THE MEDIAL PREFRONTAL CORTEX IN THE ELEVATION OF HEART RATE INDUCED BY TAIL PINCH IN THE RAT. D. Fung* and J. Stewart. Center for Studies in Behavioral Neurobiology. Dept. Psychology, Concordia University, Montreal, Canada, H3G 1M8

The noradrenaline (NA)-containing projections to the medial prefrontal cortex (MFC) are activated in response to stress. The functional role of MFC NA release in the stress-induced activation of the autonomic nervous system (ANS), however, remains undefined. This study examined the effects of microinjections of Nα-blocking drugs into the MFC on a response mediated by ANS activation, the increase in heart rate (HR) induced by tail pinch. Male rats anesthetised with urethane (1.5 g/kg) received 2-10 s tail pinches separated by 5 minutes prior to and after infusions of 10 nmol of the beta adrenergic agonist isoproterenol (ISO) unilaterally into the right MFC. Tail pinch elevated HR, which returned to basal levels in the succeeding 5 minutes. Injection of ISO into the MFC increased baseline HR, and potentiated the increases in heart rate induced by tail pinch. The alpha adrenergic agonist phenylpropanolamine (20 nmol) was without effect on either baseline HR or tail pinch induced increases in HR. These results suggest that NA released in the MFC, via stimulation of beta adrenergic receptors, plays a facilitating role in the stress-induced activation of the ANS. Studies are in progress to examine the function of the dopaminergic projection to the MFC in stress responses mediated by the ANS.

590.9 EFFECTS OF CHLORZAPAXIDE ON FOOTSHOCK-INDUCED OVERFLOW OF CEREBRAL NOREPINEPHRINE. Artur H. Swierget*, Zhongyou Wei, Yashik Li and Adrian J. Dunn. Department of Pharmacology, Louisiana State Univ. Med. Ctr., Shreveport, LA 71130-3935

Synaptic release of central catecholamines is increased during stress. The effects of benzdiazepines on the stress-related release of catecholamines has been evaluated. We have used microdialysis to examine the effect of chlorzepaxide (CDP) pretreatment on the footshock-induced release of norepinephrine (NE). Freezly moving rats were implanted with microdialysis probes in the hypothalamus and the medial prefrontal cortex (PFCM). Footshock (50 x 0.1-0.2 mA shocks over 20 min) significantly increased microdialysate concentrations of NE in the first sample collected. The subsequent two samples showed small elevations that were not statistically significant. In both the hypothalamus and the PFCM samples, NE was significantly augmented over the prefootshock baseline. CDP administration (5 mg/kg ip) had no statistically significant effects on the basal dialysate concentrations of NE, although there was a tendency towards a reduction. CDP administered before the footshock significantly attenuated the dialysate concentrations of NE.

These results suggest that footshock increases the synaptic release of NE in the cortex and hypothalamus, and that this response is attenuated by CDP. The experimental design used cannot determine whether systemic CDP alters the input to LC noradrenergic neurons, or whether benzdiazepines exert a direct effect on the noradrenergic neurons.

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590.11 FLUOXETINE TREATMENT ATTENUATES STRESS-INDUCED NORADRENAL (NE) EFFLUX IN THE HIPPOCAMPUS M.E. Page* and E.D. Abercrombie. Center for Molecular & Behavioral Neurosciences, Rutgers University, New Brunswick, NJ 08902

The noradrenergic locus coeruleus (LC) has long been implicated in various aspects of the regulation of behavioral state. This system is responsive to a wide variety of stimuli, especially novel or threatening and stress. Noradrenergic (NE) turnover increases in LC target regions in response to stress. It has been previously shown that presentation of acute stressors increases the synthesis and release of NE in the hippocampus (Abel et al., 1988). Despite its thought to contribute to a number of mental health problems including depression, anxiety and panic disorder. Chronic treatment with antidepressants alleviates many of the symptoms of depression and anxiety shows to alter LC function. However the mechanism of the therapeutic action of these drugs is unclear. It has been demonstrated that serotonin selectively attenuates LC responses evoked by local application of glutamate or nociceptive stimuli. The effect of acute treatment (2 days: 10 mg/kg/day) with the antidepressant fluoxetine, a selective 5-HT reuptake inhibitor, on hippocampal NE and DOPAC efflux was examined using in vivo microdialysis. Because there is no significant DA innervation of the hippocampus, DOPAC levels in this region are thought to reflect NE synthesis. Basal levels of NE and DOPAC did not differ between vehicle controls and 2-day fluoxetine-treated animals. Application of a clump to the rat's tail for 30 min, evoked an 80% increase in NE in vehicle control animals (n=3) and a 33% increase in animals which received FLU for 2 days (n=5). Taelpich evoked an 89% increase in extracellular DOPAC levels in vehicle animals compared to a 33% increase in FLU animals. Experiments are presently underway to determine the effects of chronic (14 day) administration of FLU on stress-induced NE efflux. One function of fluoxetine may be to modulate stress-induced responses of the LC-NE system. This work supported by NIMH DA08986.


Results from a variety of experimental paradigms have pointed to a cholinergic involvement in the stress response. Recently, analytical techniques have become available to measure ACh release in vivo during exposure to various stressors. In these experiments designed to monitor ACh output every 15 min in the dorsal hippocampus (HIPP), amygdala (AMY), nucleus accumbens and prefrontal cortex (PFC) before, during and after a 15 min session of intermittent tail shock (1/min, 1 mA, 1 sec duration) in rats. In response to the stressor, ACh release was significantly increased in the PFC (180%; p<.01), HIPP (170%; p<.01) and AMY (144%; p<.05) but not in the NAcs. In each site tested behavioral and/or maximal release occurred in the 15 min sample following release from restraint at which point ACh levels reached significance in the NAc (130%; p<.05). These data demonstrate an enhancement of cholinergic activity during application of the stressor in three ACh projection systems (HIPP, AMY, PFC).

Supported by USPHS grant NS 30697 to BGH; McDonald/Pew and Whitewall Foundations and ONR to TJS.

590.13 CHRONIC COLD STRESS ENHANCES THE RESPONSE OF LOCUS COERULEUS NEURONS TO STIMULATION OF NUCLEUS PARAGIGANTOCELULARIS M. Mame* and A.A. Grau. Dept. of Neuroscience & Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260

We have previously demonstrated that chronic cold stress increases the footshock-evoked response of locus coeruleus (LC) neurons in the anesthetized rat. Using in vivo extracellular single-unit recordings, we now demonstrate that this stress-related enhancement of evoked activity also occurs in response to direct electrical stimulation of the nucleus paragigantocellularis (nPGi), the major glomerular afferent to the LC. Twenty-four hours after termination of chronic cold stress (5°C for 17-22 days), LC neurons from cold-stressed rats displayed a significant elevation in basal firing rate (mean = 2.7 Hz for Cold Stress vs. 1.8 Hz for Controls); these basal rates were significantly higher than we have previously reported and may be related to the implantation of the stimulation electrode into nPGi. There were no differences in the threshold for activation of LC cells by nPGi stimulation (Cold Stress = 190 ± 9 μA; Control = 155 ± 4 μA in extracellular current) or in their response latencies (Cold Stress = 0.013 ms vs. Control = 0.014 ms). However, the incidence of spike activation by nPGi stimulation was greater in LC cells from the cold stressed rats (Cold Stress = 40 responses vs. Control = 34 responses; 30 stimulations at 150% of threshold), as was the incidence of burst firing (Cold Stress = 8 bursts/30 stimulations vs. Control = 4 bursts/30 stimulations). In addition, the duration of postactivation spike suppression was shorter for LC cells from cold stressed rats (Cold Stress = 600 ms vs. Control = 780 ms). We are currently investigating whether these stress-induced changes in the LC response to glutamatergic excitation from nPGi are due to changes in their sensitivity to glutamate or to a change in some other system which modulates this response.

Supported by USPHS MH 49474 and MRC Canada Postdoctoral Fellowship (MMJ).

590.15 ALtered EXPRESSION OF mRNAs ENCODING GABA A RECEPTOR SUBUNITS α1 AND α2 FOLLOWING RESTRAINT OF JUVENILE AND ADULT RATS. Roberts, Alice A; Pleger, Glorieta L; Jones, Amy W; and Kellnace, Carol K. Dept. of Psychology, Univ. of Rochester, New York, 14627.

The GABA A receptor, a multimeric protein complex located on neuronal cell membranes, mediates inhibition in cortical circuits by altering GABA-gated chloride permeability. Cortical GABA A receptors of male rats functionally respond to acute stressors in young adult (P70-90) but not at juvenile (P35) or adolecent (P35-42) ages. Likewise, young adult males exhibit altered behavioral and GABA A responsive responses based on prior experience, whereas juvenile do not. We have found modest increases (30-40%) in cortical mRNAs encoding α1 and α2 GABA A subunits immediately after two-hour restraint of young adult, but not juvenile, brains. The present study tracks expression of GABA A subunit mRNAs in young adult and juvenile brains taken at 0, 2, 4, and 6 hours following two-hour restraint; levels in cortex, hippocampus, and cerebellum, are determined by RNAase Protection Assay. Our initial results (n = 6) suggest that the impact of experience on cortical GABA Aα subunit expression may develop across adolescence in parallel with functional responsiveness of cortical GABA A receptors to acute stressors. Since juveniles lack experience-dependent behavioral responses, it may be that altered GABA Aα subunit expression underlies inhibitory synaptic plasticity critical for specific behavioral adaptation to challenging environmental stimuli.

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590.17


Exposure to toxic stressors such as noxious tailshock (IS) lead to a variety of behavioral alterations not produced by controllable stressors. While noxious stressors that induce effects
because IS leads to an intense activation of the dorsal raphe nucleus (DRN), a consequent downregulation of inhibitory somatodendric 5-HT1A autoreceptors, and hyper-excitability of the 5-HT1A receptors.

The present experiments tested these possibilities by inhibiting DRN activity either before IS exposure or before behavioral testing 24 hr later. By the above arguments, one should block the behavioral effects of IS. Male Sprague-Dawley rats were either exposed to 100 unignited 1 mA tailshocks or were loosely restrained for an equal time period. Testing occurred 24 hr after shock or restraint and involved fear conditioning and shuttlebox escape testing. The 5-HT1A agonist 8-OH-DPAT was infused into the region of the DRN (1 ug in 1 ul) either before the IS treatment or prior to testing. These results differ from those reported for systemic administrations of 8-OH-DPAT. However, doses of systemic 8-OH-DPAT may facilitate rather than inhibit 5-HT activity through action at post-synaptic receptors. Therefore, different doses of 8-OH-DPAT were systemically administered before testing. A dose of 0.1 mg/kg blocked the enhanced escape deficit produced by IS, 0.1 mg/kg attenuated this effect of IS, and 1.0 mg/kg had no effect on escape performance. Conditioned fear, however, was blocked by all doses of 8-OH-DPAT. Support provided by NIH MH 50479.

590.18

THE EFFECTS OF SOCIAL STRESS ON SEROTONERGIC INDICES IN PREFRONTAL CORTEX OF ADULT MALE CYMOLOUS MONKEYS. MB Rochelot*, JR Kaplan, SR Mannik, JF Mann, Dept. of Comparative Med., Bowman Gray Sch. of Med., Wake Forest Univ. Winston-Salem, NC 27117 and Departments of Psychology and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15213.

The effects of social stress and maternal status on monoamine concentrations in the prefrontal cortex were examined in adult male cynomolgus monkeys (Macaca fuscata). Seventy-five animals were housed in five-member social groups for 28 months. Social groups either remained intact at the time of testing or had their group membership (no-stress condition) or had their group membership reorganized at monthly intervals during months 1-14 (post-stress condition) or during months 15-28 (recent-stress condition). At necropsy, brain samples were collected and stored at -70°C until analyzed. Frontal pole cortical and CSF monoamines (serotonin [5-HT], dopamine, noradrenaline and metabolites [5-hydroxyindoleacetic acid [5-HIAA], homocysteic acid, 3-methoxy-4-hydroxyphenylglycol [MHPG]) were assayed using high-performance liquid chromatography with electrochemical detection.

Animals exposed to past social stress had significantly lower 5-HT (p < 0.02) and 5-HIAA (p < 0.01) concentrations in the prefrontal cortex compared to animals in the no-stress condition. Prefrontal cortical 5-HIAA and 5-HT concentrations in the recent-stress condition were intermediate between the concentrations in the no- and post-stress conditions. These data suggest that past exposure to chronic social stress may be associated with an enduring and perhaps progressive reduction in serotonergic function in the prefrontal cortex. Such an effect may underlie the behavioral sequelae of stress such as post-traumatic stress disorder.
HORMONAL CONTROL OF REPRODUCTIVE BEHAVIOR: PARENTAL/AGGRESSIVE

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THURSDAY AM

95.1
EXCITOTOXIC LESIONS OF THE VENTRAL BED NUCLEUS OF THE STRIA TERMINALIS DISRUPT MATERNAL BEHAVIOR IN RATS.
M. Numan, M. Jung, J. Psychology, Boston College, Chestnut Hill, MA 02167.
Lesions of the medial preoptic area (MPOA) severely disrupt maternal behavior in rats, but these lesions also damage the ventral part of the bed nucleus of the stria terminalis (VBNST). Other work has shown that Fos expression increases in both the MPOA and the VBNST of lactating maternal females rats. Finally, anatomical work has suggested that these lesion may be VBNST efferents to the brainstem, rather than MPOA efferents, which are important for maternal behavior.

The present work directly tests the importance of the VBNST. Pregnant rats with pretreatment received one of the following: bilateral injections (3 ug/ul) of the excitotoxin, N-methyl-D-aspartic acid (NMDA) directly into either the VBNST or the doratal BST, or bilateral injections of the inactive, I-aminoacid, into the VBNST. Only animals that received NMDA injections into the VBNST showed severe deficits in maternal behavior.

These results suggest that VBNST efferents are important for maternal behavior. Additional work is iontophoretically applying PHA-L to the VBNST in order to examine its major projection routes and termination sites.

Supported by NSF Grant 19831315.

95.1.7
THE DISTRIBUTION OF PROLACTIN BINDING SITES IN THE BRAIN OF BROODING SALAMANDERS.
L.P. Mangurian*, K.I. MacArthur, R.L. Sejnowski, and D.C. Forester. Department of Biological Sciences, Towson State University, Towson, MD 21204.
Prolactin (PRL) influences reproductive behaviors and has been shown to stimulate maternal behavior in rats and doves. The reproductive behavior of amphibia, such as the water drive prior to oviposition, is also influenced by PRL. Desmognathus fuscus females exhibit a brooding behavior. This study was designed to investigate the possibility that prolactin may influence brooding behavior by examining their brain potential prolactin sensitive areas. The localization of PRL target areas was carried out using in vitro autoradiography. Frozen brain sections were incubated with 125I ovine PRL and then autoradiographed. Only the olfactory bulb (300 fold) regions were found.

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95.1.9
QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS OF D1 AND D2 DOPAMINE RECEPTORS IN RAT BRAIN ACROSS PREGNANCY.
Previous work has suggested that dopamine may be a neurotransmitter involved in some aspects of maternal behavior (Phys & Behav 48:211). In addition, treatment of females with pharmacological doses of dopamine, a hormone important in late pregnancy, increased the level of dopamine binding in the striatum (Brain Res 46:249, 1988). We hypothesized that the naturally occurring physiological and endocrine events of pregnancy may alter the level of the D1 and D2 dopamine receptors in a regional specific manner. Using quantitative autoradiography (D1 receptors: 1.0 mg/mL-C-3H-SCH-23390; D2 receptors: 1.0 mg/mL-D-2-spiroperidol), brains from pregnant females (day 1 to 21), non-pregnant females, and males were examined. As predicted from previous work, even in pregnant females, in most brain regions D1 receptors were present in greater density than D2 receptors. The highest levels of D1 and D2 binding were in striatum, nucleus accumbens, and olfactory tubercle. Moderate to low levels of D1 binding were found in zona incerta, thalamus and hypothalamus, and low levels of D2 binding were found in olfactory bulb and hypothalamus. In some brain regions, pregnancy did not alter the D1 or D2 receptor levels; in other brain regions receptor binding was significantly different in females on day 2 compared to day 21 of pregnancy. For example, D2 females had substantially lower levels of D1 receptor in lateral and medial striatum than 2 day pregnant females. Preliminary results also show that late pregnant females had modestly lower levels of D2 receptors in anterior striatum, nucleus accumbens and olfactory tubercle. These results may provide clues to the brain regions that are most important for dopamine mediation of maternal behavior in rats. Supported by HD 22963 to JBM.

95.1.6
CHOLECYSTOKININ (CCK) AND THE REGULATION OF MATERNAL BEHAVIOR.
Recent work in our laboratory has established that concurrent intracerebroventricular (icv) infusions of CCK-8 with -endorphin prevents the disruptive effects of -endorphin on the maintenance of maternal behavior in lactating rats (Felicio et al., 1991). CCK has also been shown to stimulate the onset of maternal behavior in virgin rats; Linden et al. (1985) found that chronic pretreatment with CCK-8 (300 pg) for 12 h stimulated maternal behavior within 2 hours of exposure to pups. The goal of the present series of experiments was to further examine the role of CCK in the induction of maternal behavior and characterize CCK's involvement in ongoing maternal behavior. In a set of studies evaluating the role of CCK in the onset of maternal behavior, we were unable to stimulate maternal behavior in virgin Sprague-Dawley rats treated chronically with CCK-8 (300 pg) or in steroid-primed, ovariectomized, virgin rats given icv infusions of CCK-8. Moreover, chronic infusions of CCK-8 into the lateral ventricle of primigravid rats late in pregnancy also failed to stimulate maternal behavior. In contrast to the apparent lack of involvement of CCK in the onset of maternal behavior, ongoing maternal behavior appears to be regulated in part by CCK. When lactating rats were infused icv on day 5 postpartum with either 25 or 126 pg of progesterone, a nonspecific CCK antagonist, lactating rats displayed longer latencies to first crouch over their young, an effect similar to the inhibitory response found after central infusions of -endorphin (Mann et al., 1991,1992). Together, these data suggest to us that CCK-8 is more strongly involved in the regulation of ongoing maternal behavior than in the induction of maternal behavior in the rat. Further studies are needed to determine sites and modes of CCK action. Supported by PHS Grants DA04291 and HD19789 (RSB).

95.1.8
FOS IMMUNOREACTIVITY IN THE HYPOTHALAMUS ASSOCIATED WITH PARENTAL BEHAVIOR IN THE PRAIRIE VOLE, M. OCHROGASTER. B. Kirkpatrick*, D. Litman, T.W. Kim. Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD 21228.
A number of studies have implicated the paraventricular nucleus of the hypothalamus (PVN) in the onset of maternal behavior in rats. However, we found no increase in Fos immunoactivity associated with maternal or female parental behavior in the PVN in the prairie vole, a species in which both males and females spontaneously exhibit parental behavior prior to sexual maturity.

To clarify the role of hypothalamic Fos in paternal behavior, we examined cellular activation in areas of the hypothalamus other than the PVN, following exposure to a pup in male prairie voles. Fos immunoactivity was quantified in suprachiasmatic, ventromedial, dorsomedial, and premammary nuclei, and the anterior and posterior hypothalamic areas. Compared to controls exposed to a novel olfactory stimulus, males exposed to pups showed an increase in several discrete areas. These results suggest hypothalamic areas other than the PVN may be involved in the control of paternal behavior in this species.

95.1.10
EFFECTS OF FOOD RESTRICTION ON MONOAMINE CONCENTRATIONS IN HYPOTHALAMIC NUCLEI OF THE CHICKEN BRAIN. L. Moors,* Y. Gu and F. Vandesande* Lab. of Neuroendocrinology, Zoological Institute, Naamsestraat 59, 3000 Leuven, Belgium, Europe.
Intensive selection for increased muscle growth in meat-type chickens has led to a decrease in the reproductive performance. Excessive food restriction has become the standard industrial procedure to improve the reproductive efficiency of the meat-type parent stocks. The neural control of both the feeding and the reproductive behavior seems to happen through identical neurotransmitters, catecholamines and serotonin. In order to determine whether food restriction alters in some way the concentration of monoamines in hypothalamic nuclei assumed to be involved in the control of reproduction, - ad libitum fed and food restricted female meat-type chickens were killed at the ages of 4, 10, 16 and 22 weeks. A quantitative determination of the concentration of biogenic amines was performed in ten different micropunctured hypothalamic brain areas. The amines: L-DOPA, dopamine, norepinephrine (NE), epinephrine and serotonin and their major metabolites were separated on a C-18 HPLC column and determined by electrochemical detection. In most hypothalamic areas studied and for most neurotransmitters determined no concentration difference, resulting from food restriction, was found. However, food restriction did affect the amount of certain biogenic amines in a few hypothalamic nuclei. The most striking difference occurs in the median eminence, where the concentration of NE is largely increased in food restricted animals. These quantitative data provide an excellent basis for further research on the possible role of catecholamines and indolamines in the altered feeding and reproductive behavior in the meat-type chicken.
EPILEPSY: MECHANISMS OF EXCESSIVE DISCHARGE

592.1

NPY, SS, AND GAD MOLAR MOLECULAR LAYER SPROUTING IN HUMAN EPILEPTIC HIPPOCAMPI. G.W. Mathern, T.L. Babb, J.L. Leite, J.K. Preoty, and J. Engel, Jr. UCLA School of Medicine, Los Angeles, CA 90024-1766.

Patients with Hippocampal Scarring (HS, n=18), Mass Lesions (Mass, n=9), or autopsies were compared for differences in: 1) percentage of hilar neurons immunoreactive (IR) for NPY, somatostatin (SS), and GAD, and 2) patterns of fascia dentata (FD) IR-sprouting, and FD densities of FD IR-sprouting. Results were: 1) NPY-IR hilar neurons decreased in an equal proportion to all hilar neurons (p=0.47), SS-IR neurons were significantly decreased in HS in excess of hilar n=0.03), and GAD-IR neurons were not significantly different (p=0.26). 2) Outer molecular NPY-IR decreased in HS compared to Mass (p=0.0002), but was unchanged in SS-IR (p=0.09), and inner molecular layer with GAD-IR (p=0.007). 3) The patterns of IR-sprouting between HS and Mass were significantly different (p=0.001). This supports the notion that peptide hilar counts and molecular layer synaptic reorganization differ among peptides and between patients with HS and Mass pathogenic mechanisms. Supported by NS 20808 and KO8-NS 01603.

592.2


Although status epilepticus (SE)-induced brain damage is well-documented in animal models, human cases of neuronal damage caused strictly by SE have been difficult to document because of systemic factors or a history of pre-existing epilepsy. It has been hypothesized that excessive release of neurotransmitter glutamate is responsible for the SE-induced neuronal necrosis. We report 3 patients who died 12-27 d after the onset of focal motor SE, in whom hypotension, hypoxemia and hypoglycemia do not complicate the interpretation of results. Two of the 3 patients had no prior seizures and no known cause of their SE. The third patient, who had leptome ningeal carcinomatosis, had 1 seizure 2 months before the onset of SE. The duration of SE was 2.5 h-3 d. EEGs showed unilateral temporal lobe sharp-wave discharges in 1 and independent temporal lobe sharp-wave discharges bilaterally in the other 2. There was widespread neuronal loss in the hippocampus (CA1-3 and hilus), amygdala, piriform cortex, dorsomedial thalamic nucleus and cerebellum (Furkine ecells), with scattered neuronal loss in the cerebral cortex. In 2 of the 3 cases the damage was unilateral and occurred on the side in which seizure discharges were most prominent. The regional distribution of the damage in these 3 cases was similar to that found in animal models of limbic SE: it occurred primarily in limbic structures with high densities of glutamate receptors. (Supported by the Dept. of Veterans Affairs.)

592.3


In order to investigate the prevalence of neuronal synchronization in specific pathways of the human hippocampal formation and amygdala, EEG spikes, single and multiple cell action potentials were recorded in patients with surgically implanted intracranial depth electrodes required for diagnostic studies. MRI guidance was used to place electrodes bilaterally in amygdala, anterior and medial hippocampus, entorhinal cortex, and parahippocampal gyrus. Bundles of 9 microwires were used for extracellular recording. In one group of patients interictal EEG spikes were monitored continuously for 6 to 10 and bipolar spike density maps were obtained at each recording site using EEG spike detection software. Spike frequency distributions (spikes/min) were plotted over the entire period and crosscorrelations between recording sites were computed. Although correlations varied widely, a direct relationship was found between spike rate and the probability of a significant positive correlation between specific anatomical pathways, including the periforn, longitudinal association, and retrohippocampal pathways. In a second group of patients, microelectrode recordings 5 to 30 min in duration were obtained from a total of 255 cells and subjected to crosscorrelation analysis between 2 to 8 simultaneously recorded cells. Or 93 possible within-bundle unit correlations, 49% were significant, and of 516 possible between-bundle correlations dependent upon anatomical connections, 25% were significant. Both showed a greater proportion of significant correlations in the temporal lobe where seizures originated. Supported by NS 20808.

592.4


Synchronized neuronal burst discharge is hypothesized to initiate ictal events. If so, inter-ictal neuronal synchrony in epileptogenic structures may include a higher percentage of burst action potentials (burst APs). We therefore sought to examine the relative contributions of isolated and burst APs to synchronous interactions both ipsilateral and contralateral to seizure onset in patients with complex partial seizures. Spontaneous inter-ictal single cell activity was recorded from the hippocampus, amygdala, and other temporal lobe structures of patients undergoing chronic depth electrode implantation to localize seizure onset. When cross-correlation histograms between pairs of simultaneously recorded cells contained a significant central peak, implying a synchronous interaction between those cells (r=0.75 ipsilateral, r=0.93 contralateral), the proportion of burst APs contributing to that peak was calculated for each cell. Bursts APs showed a strong trend towards a proportionally greater contribution to synchronous interactions contralateral to the side of seizure onset. The correlation between the likelihood of a neuron to discharge bursts and the contribution of burst APs to synchronous interactions involving that cell was stronger in the contralateral hemisphere (r=0.72) than the ipsilateral (r=0.72). Our results suggest that in the inter-ictal state, regions that commonly initiate seizures are less likely to contain synchronously bursting neurons. Supported by NS 20808.

592.5


Modulation of neuronal discharge by the cardiac cycle is relatively common in the mammalian nervous systems. Cardiac cells in amygdala is markedly higher ipsilateral to seizure onset, suggesting an interaction of these cells with epileptogenesis. We examined 314 single units from patients with epilepsy undergoing depth recordings of mesial temporal EEG. Bilateral recordings were performed in amygdala, hippocampus, anterior and posterior perihippocampal cortex, and entorhinal cortex. From 3 to 20 spike trains were recorded simultaneously from each patient, permitting direct evaluation of the nature and number of cell interactions. Spike trains were characterized on the basis of cross- and auto-correlograms, with inhibitory and excitatory phenomena recorded as the proportion of possible interactions. Cardiac-modulated cells were more likely to participate in spike trains and were strongly coupled with inhibitory interactions. In amygdala, this relationship was strikingly dependent on the side of seizure onset. Cardiac-related amygdala cells on the contralateral side participated in inhibitory interactions, and were likely to "drive" cells in other structures. On the ipsilateral side, cardiac dependency was associated only with a bursting pattern of discharge. This difference may result from a loss of region inhibitory connections to cardiac-dependent cells. Supported by NS 20808.

592.6


Paced-pulse stimulation of the perforant pathway has been used to animal models of epilepsy to examine the status of inhibitory processes in the dentate granule cells, relative facilitation of the granule by observed following experimental treatments which destroy inhibitory interneurons in the dentate hilus1. We report the intraoperative use of the paired-pulse paradigm in eighteen patients undergoing synchronous for medically intractable seizures. During the resection surgery, after the lateral leucocortex, monophasic depth electrodes were placedcephalad through the dentate gyrus. Four contact strip electrodes were also placed on the hippocampal alveus surface and on the entorhinal cortex. Stimulation to the perforant path was performed utilizing single pulse paired pulses with interpulse intervals of 20, 40, 60-100 and 200 msec. In the initial 10 patients, PSs were recorded without modulation spike. Technical improvements have allowed us to record clear population spikes in six of the last 8 patients. Inhibition was observed in the pairing of the PSP or spike amplitude selected at each IPI to that measured after a single pulse. In an on-going study, these measures are compared to pathological studies of the excised hippocampus, other in-vivo and in-silico electrophysiological studies, and the test predicted in part by an American Heart Assoc. Fellowship and NIH Grant NS06169)

592.7

DISTRIBUTION OF CALCIUM-BINDING PROTEINS PARVALBUMIN, CALBINDIN D-28K, CALRETININ, AND PERINEURONAL NETS IN THE HUMAN EPILEPTIC HIPPOCAMPUS. C. Eckedo*, I. Slivkina*, M. R. Cela*, C. E. Crawford, and J. D. White. Dept. of Neurosurgery, University of Bonn Medical Center, 53105 Bonn, Germany. 2 Dept. of Histology and General Embryology, University of Fribourg, 1705 Fribourg, Switzerland. Neural vascular changes during epileptic seizures has been associated with an increase in intracellular free calcium concentration. Hippocampal neurons which contain the calcium-binding proteins (CalpBPs) parvalbumin (PV), calbindin D-28K (CB) and calretinin (CR) are, therefore, reasonable candidates for studies on the morphological and neurochemical alterations in human temporal lobe epilepsy (TLE). PV+ and CB+ neurons (PV-Lo) neurons are also covered with different extracellular matrix molecules forming the perineuronal nets (PNs) and are surrounded by astroglial processes. PNs may protect these neurons from an excess of extracellular glutamate during seizures.

We studied the distribution of PV-Lo, CB-Lo, and CR-Lo neurons in hippocampus (HC) specimens obtained from patients with TLE. Autopsy brains were used as controls. The immunohistochemical staining pattern were correlated with the following histopathological changes: (1) HC without histological alterations; (2) HC with focal lesions or tumors; (3) HC with Ammonshorn sclerosis (AS); (4) HC with both, focal lesions/tumors and AS. In general, the neuronal loss observed in TLE patients was associated with a decrease in CB+ neurons. However, among the surviving neurons, many PV+ and CB-Lo neurons were covered with PNs. In some patients, CR-Lo showed an increase in neurileptol staining in the dentate gyrus and CA3 region, as well as "de-novo" granule cell staining in some of the CR-Lo neurons were covered with PNs. We conclude that neurons expressing PV and CB, which were surrounded by PNs, were less vulnerable to degeneration in TLE. In addition, CR may serve as an interesting marker for activated and reorganizing neuronal systems in human TLE.

Supported by the German BMFT Helmholtz-fellowship for I.B.

592.9

MORPHOLOGY OF GRANULAR NEURONS IN THE HUMAN HIPPOCAMPUS. G. von Campe, P. Baard* D. Spencer, N. C. de Leraovere, Yale University School of Medicine, Section of Neuropsychology, New Haven, CT 06519.

Examination of hippocampi removed from patients with medically intractable temporal lobe epilepsy (TLE) has shown considerable neuronal reorganization within the seizure focus. Granule cells and their mossy fiber axons contain the opioid peptide dynorphin, which in normal hippocampi is not expressed in the molecular layer. In patients with mesial temporal sclerosis, a dense band of staining is observed in the inner molecular layer, suggesting collateral sprouts from granule cells into the molecular layer. To directly examine the presence of such collaterals from granule cells we studied the morphology of individual cells by using an intracellular injection technique. Fluorescent microspheres (4%) fixed with formalin were sliced into 100 μm thick sections on a Vibriostat. Individual neurons were then visualised and photographed (20x for 5-15 minutes) with Lucifer Yellow (3-5% aqueous solution) under UV illumination. Selected filled cells were then photoinjected with UV irradiation in the presence of diamobenzidine (DAB) to produce a peroxidase-like reaction. In patients with mesial temporal sclerosis at least two morphological types of granule cells were found. One type was a single branched apical dendrite and a single axon-like process extending from the opposite end toward the hilus. The other type of granule cell has a more rounded cell body with two to several apical dendrites and a single basal axon. In several neurons, fiber-like processes were observed to arise from the basal to lateral region of the cell body, and after a short course toward the hilus, they curved back up and entered the molecular layer. Such processes may be the morphological basis of the dynorphin immunoreactivity observed in the inner molecular layer of hippocampus from patients with temporal lobe epilepsy. Supported by NS06208.

592.11


Glutamate is the principal neurotransmitter identified within the intrinsic circuitry of the hippocampal formation. It is hypothesized that glutamate plays a central role in excitotoxicity associated with temporal lobe epilepsy. In this report we have addressed the organization of the AMPA receptor subunits within this hippocampal circuitry by immunocytochemical localization of the AMPA selective subunits GluR1 and GluR2/3 in relation to patterns of cell loss in patients with temporal lobe epilepsy.

Hippocampal tissue was resected during surgery from patients with medically intractable temporal lobe epilepsy. Representative sections of tissue were placed in 4% paraformaldehyde with 0.2% picric acid. Serial sections were cut either on the vibratome or freezing microtome and stored in a cryoprotectant solution. Tissue was processed immunocytochemically using antibodies generated against the GluR1 subunit and the GluR2/3 subunits.

The localization of these subunits is distinct in the hippocampus. The GluR1 subunit is restricted to fibers of the molecular layer of the dentate gyrus, whereas GluR2/3 subunits' distribution is widespread. Surviving pyramidal cells in all CA subfields stain positively for GluR2/3. In addition, immunoreactive neurons are also present throughout the dentate gyrus. Knowledge of the distribution of selective glutamate receptor subunits can elucidate the effects of cortical injury that may proceed in temporal lobe epilepsy. Supported by NIH grant NS21323.

592.8

GABA-A ALPHA AND BETA RECEPTORS AND GABAA AXON SPROUTING IN HUMAN HIPPOCAMPAL EPILEPSY. T.L. Babb*, G.W. Mathern, J.P. Lete, and J.K. Frickhinger. UCLA School of Medicine, Los Angeles, CA 90024-1769.

Resected human hippocampi (HC) with Hippocampal Sclerosis (HS; n=6) or little HC cell damage (n=4), a large mass lesion and a HC mass lesion (n=2) were compared for GABAergic (GAD-ICC) vs control (FD) (MD) axon sprouting and "matching" changes in post-synaptic GABA-A receptor subtypes. Neuronal and axonal changes in the FD-M were weakly correlated with cell losses in the HP (p=0.07) and FD (p=0.07; i.e. reactive synaptogenesis). The densities of FD-ML receptor subtypes for GABA-A alpha (p=0.28) and beta (p=0.85) did not correlate with neuron loss (i.e. HS). There was a trend for the GABA-A receptor subtypes to have similar densities in the FD-ML (p=0.08) suggesting that both beta types were similarly affected in epileptic FD. Finally, the GABA axon patterns in the FD-ML scored semi-quantitatively, showed varying densities and distributions across the inner through outer molecular layers, and these did not predict GABA-A alpha (p=0.15) or beta (p=0.70) densities, suggesting that in epilepsy there may be heterogeneous remodelling of receptor subtype patterns to aberrant presynaptic terminals. Supported by NS 20808, NIH-FIC, CNPq (Brazil) and K08-NS 061605.

592.10

ANALYSIS OF HUMAN EPILEPSY-ASSOCIATED GENE EXPRESSION BY MESSANGER RNA DIFFERENTIAL DISPLAY. H. Xie, M. L. Brines, J. K. Kim*, N. C. de Leraovere. Sections of Neuropsychology, Neuropathology, and Neuroendocrine Program, Yale University School of Medicine, New Haven, CT 06519.

Human diseases (including temporal lobe epilepsy; TLE) are usually associated with alterations in gene expression. These changes in gene regulation may be the consequence or the cause of the underlying disease. Detailed study of the alterations in patterns of gene expression could provide insight into the etiology of the disease. The objective of this study is to identify genes which are differentially expressed in one subgroup of epilepsy characterized by mesial temporal sclerosis and associated hippocampal reorganization (MTLE), compared to another subgroup characterized by extrahippocampal temporal or neocortical lesions (ExH group). We used mRNA differential display methodology, which involves the reverse transcription of mRNA into cDNA and the use of PCR with random primers to amplify the cDNA signals. We applied the differential display methodology to two pairs of hippocampal specimens obtained from patients operated to control TLE. More than eighty different primer pairs were used for the PCR differential display, which statistically should cover almost all mRNA species. Fifty-two uniquely amplified bands were identified and the corresponding DNA fragment extracted from the dried sequencing gels. Five of the 52 bands have been closed and sequenced to date. Four of them have no matches in the database GenBank, which indicates that those sequences may represent novel mRNA species yet to be characterized. The fifth cDNA fragment is 320 bases long, with a 70% homology (in a 150 bp overlap region) to the innervate transposase gene mariner. Our preliminary PCR, Northern, and Southern blot data indicate that this 320-base sequence is represented in the normal human genome. However, the level of expression in normal humans is reduced compared to epilepsy patients. Further characterization of this gene is in progress. We conclude that mRNA differential display is highly efficient in identification of disease specific genes and in the analysis of gene expression alterations in human epilepsy brain tissue. Supported by NS 27081.

592.12

HIPPOCAMPAL NMDA RECEPTOR NR1 SUBUNIT EXPRESSION IN EPILEPTIC HUMAN HIPPOCAMPUS. I. Nunn*, G. Tooco, V. Comar, R. Kakagi, H. Liiders, and M. Bandeir. Section of Epilepsy Surgery, The Cleveland Clinic, Cleveland, OH, and Neurosciences Program, USC, Los Angeles, CA.

NMDA receptors, subtype of glutamate receptors, have been shown to play an important role in the genesis of seizures. The NR1 subunit of the NMDA receptor is associated with increased neuronal excitability in temporal lobe epilepsy (TLE). We examined the changes in the prevalence of the NR1 subunit mRNA of the NMDA receptor in frontal and temporal lobes of patients with temporal lobe resections in patients with intractable TLE (n=12). In situ hybridizations using a labeled oligonucleotide recognizing the NR1 subunit mRNA of the NMDA receptor were used to generate autoradiograms. Optical density measurements were performed in different hippocampal subfields. Our results show significant increase in NR1 subunit expression in both control and astroglial type immunoreactivity was observed across the hippocampal formations from patients suffering from intractable TLE as compared to control hippocampal tissue obtained at autopsy. The increased expression of NMDA receptor subunit mRNA in hippocampal tissue was apparent in contrast with a previous report showing a decrease in total NMDA receptor/channel binding. This may represent a preferential expression of certain subtypes of NMDA receptors that may explain the apparent variability and participate in the genesis of seizures in patients with TLE. Supported by grant NIH 510377 from NSF (MB).

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994 THURSDAYAM
592.13 IMMUNOHISTOCHEMICAL DISTRIBUTION OF NMDA-RECEPTOR (R1), AMPA-RECEPTOR (R2), AND KAINATE (R5/7) RECEPTOR SUBUNITS IN HUMAN EPILEPTIC HIPPOCAMPUS. L. Blinicka, C. Eischiish, H.C. Wold, O.D. Wiessing. Dept. of Neuropathology, University of Bonn Medical Center, Sigmund-Freud Str. 25, 53105 Bonn, Germany.

Glutamate receptors and receptor-mediated excitotoxicity have recently been associated with the pathogenesis of human temporal lobe epilepsy (TLE). Using monoclonal antibodies, which selectively recognize the N-methyl-D-aspartate (NMDA) R1-receptor, the alpha-amino-3-hydroxy-5-methyl-4-oxo-pyridine (AMPA) R2-receptor and the kainate R5/7-receptor subunits (kindly provided by J.H. Morrison), we have examined the immunohistological distribution of these glutamate receptor isoforms in the hippocampal formation (HC) of patients with intractable TLE. Normal human HC-specimens revealed at autopsy were used as controls. Histopathologically, the specimens were classified into 4 categories: (1) HC without histological alterations; (2) HC with focal lesions, e.g. ganglioglioma, low-grade glioma, dysplasmoepithelial neoplasms, hamartoma or vascular malformations; (21%); (2) HC with Ammonshorn sclerosis (AS); 54%; (4) HC with both focal lesions and AS (15%). Glutamate analysis of the regional and cellular distribution of NMDA R1, AMPA GluR2, and kainate GluR5/7 immunoreactivity showed that the vast majority of neuronal cell bodies and dendrites of the granule and pyramidal cell layers exhibited immunoreactivity for all three proteins. We found no evidence for compensatory upregulation or selective loss of receptor expression in the HC of patients with chronic TLE. A decrease in or loss of immunoreactivity was closely correlated with the overall neuronal loss. So far, there was only one patient with a substantia nigra in HC R1 staining in the polymorphic layer of the dentate gyrus and in the stratum lucidum of CA3. However, this data may indicate that the glutamate receptor subunits are not directly involved in the pathogenesis of human TLE.

Supported by the German BMT-Helmholtz fellowship for I.B.


The transfer of genetic information into hippocampal cells in primary culture, slice preparations and test animals is yielding significant new information about the physiology and pathology associated with the hippocampus. In addition, gene transfer into the hippocampus provides hope for gene therapy in diseases affecting the hippocampus, including some forms of epilepsy.

We have recently developed an adenoviral-associated virus (AAV) vector containing the Lac Z gene, encoding a bacterial ?-galactosidase, under control of a cytomegalovirus immediate Early promoter. This vector and appropriate controls were injected into the hippocampal tissue derived from human patients undergoing temporal lobectomies for medically intractable seizures. Five hundred microliters contained in artificial CSF 3°C for 5 hours, and then 30 ?m thick slices were generated from the larger slices and X-gal staining was performed to ascertain the presence of the bacterial ?-galactosidase. In those slices exposed to the AAV vector with the Lac Z gene, significant ?-galactosidase activity was observed in cells surrounding the injection track. No staining was seen in controls. The implications for gene transfer transfer into the human hippocampus and other CNS locations are addressed.


Rasmussen's encephalitis is a childhood disease of intractable focal seizures and characteristic inflammatory histopathology in the affected brain hemisphere. Two rabbits injected with a bacterial fusion protein expressing neuronal receptor subunit, GluR3, were observed to develop seizures and early histopathological changes similar to those observed in Rasmussen's encephalitis. To test the hypothesis that NMDA GluR3 may be associated with Rasmussen's encephalitis, the sera from affected patients and age and sex matched controls were examined for immunoreactivity towards GluR3 subunits using Western blot analysis and transfected cells. Rasmussen patients with active disease were found to have circulating IgG antibodies to GluR3. In a therapeutic trial of one patient, removal of circulating GluR3 antibodies by plasmapheresis correlated with a reduced rate of seizure and improved cognitive function. Our results suggest that Rasmussen's encephalitis consists of an autoimmune component which includes autoantibodies towards glutamate receptors of the CNS. Supported by NIH grants NS030990, NS28709, NS17771, NS24448, EY08362 and M01-RR-30.


Paraneoplastic (PN) cerebral degeneration is a remote complication of disseminated systemic cancer. This disease is characterized by loss of Purkinje neurons, variable loss of granule cells, and occasional peripheral lymphocytic infiltrates. High titer anticerebellar antibodies are often present. We have examined a series of 16 patients with Type 1 or Type 2 PN disease for autoimmune reactivity to glutamate receptor (GluR) subunits using both Western blot and immunohistochemical analyses. We identified one patient with the Type 1 paraneoplastic disease whose serum exhibited immunoreactivity to GluR5. Unexpectedly, immunohistochemical analyses of mouse brain sections with serum from the same patient exhibited highly specific regional staining of the lateral septum, bed nucleus of the stria terminals, centromedial habenula, and dentate gyrus of R1,3,4,5 subunits in the cerebellum. Staining of this pattern persisted to dilutions of 1:20,000. This pattern of expression is strongly suggestive of an immunohistochemical phenomenon for GluR1,2,3,4,5 (Rogers, et al. JNS 11:2713, 1991). These results suggest that this patient may harbor autoantibodies to more than one GluR subunit. This is the first peptide of GluR1,2,3,4,5 subunits can occur in PD disease, and that this differs from the anti-GluR3 immunoreactivity observed in Rasmussen's encephalitis (see abstract by Rogers et al). Any of these GluR subunits may serve as autoantigens in neurodegenerative disease.

592.18 NEURONAL GLUTAMATE RECEPTOR ANTIBODIES — POTENTIAL EXCITOTOXINS? L.C. Gahing, R.E. Twyman, M.J. Eichen, S.N. Rutweks, L. Jackson, J.R. Baringer* and S.W. Rogers. Programs in Neuroscience and Human Molecular Biology & Genetics, Univ. of Utah School of Medicine and VA-GRECC, Salt Lake City, UT 84112.

Two rabbits injected with bacterially prepared protein of neuronal glutamate receptor subunit GluR3, developed autoantibodies and pathology similar to those observed in Rasmussen's encephalitis (see abstract Rogers et al). Our initial studies have focused on rabbit immune serum and the mechanism of antibody action. Rats injected with at least two epitopes exist in the region of aminocid 245-457 of GluR3 near the presumed extracellular glutamate binding site. Immunohistochemical examination on mouse brain reveals the presence of GluR3 expression consistent with in-situ hybridization studies. These antibodies also identify a small subset of morphologically similar cells in live mouse (E14) cortical neurons in culture. Whole cell electrophysiological studies of these neurons indicate that GluR3 expressed in our model system are non-NMDA sodium currents and that these neurons also have kainic acid inducible currents. Antibody evoked currents were blocked by CNQX and currents were not elicited by other sera. Single cell RT-PCR analysis of these neurons identifies the presence of GluR3 and additional GluR subunits. These results indicate that antibodies to GluR3 have antigen properties on unique properties that include neurotoxicity in conditions such as epilepsy, neurodegenerative diseases and paraneoplastic syndromes.
592.19

CHANGES IN HIPPOCAMPAL GLUTAMATE/AMPA RECEPTORS IN EPILEPTIC HUMAN HIPPOCAMPUS

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Changes in glutamate receptor properties in the limbic system have been proposed to participate in the increased neuronal excitability observed in human temporal lobe epilepsy (TLE). We examined the changes in binding properties of hippocampal glutamate/AMPA receptors using quantitative ligand binding autoradiography in frozen-sectioned sections obtained from temporal lobe resection in patients with intractable TLE. As observed in rat hippocampal sections, preincubation of human hippocampal sections (n=12) at 15°C resulted in a large decrease in [3H]-AMPA binding as compared to sections preincubated at 0°C. Preincubation at 15°C in the presence of calcium (2 mM) resulted in a large increase in binding as compared to sections that were not treated with calcium. Sectioning from epileptic patients exhibited an increase in binding following preincubation at 35°C in the absence of calcium and no change after preincubation in the presence of calcium as compared to control tissue obtained at autopsy. Our results indicate that TLE is associated with a decrease in synaptic receptors and an increase in non-synaptic (possibly cytoplasmic) AMPA receptors.

Thus, the changes in AMPA receptor binding are more complex than previously reported, and the increase in functional AMPA receptors with calcium at physiological temperature, may contribute to the hyperexcitability in epileptic human hippocampus.

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593.1

POTENTIATION OF AUDIogenic SEIZURES BY TMPp AND PTZ IN NAIVE ADULT FISCHER-344 AND SPRAGUE-DAWLEY RATS

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Trimethylpropane phosphate (TMPp) and pentyltetrazol (PTZ), two potent convulsants thought to antagonize the GABA, inhibition system, potentiated audiogenic seizures in naive adult Fischer-344 and Sprague-Dawley rats. Doses of TMPp (0.0125-0.05 mg/kg ip) insufficient to produce observable behavioral convulsions or EEG response nevertheless potentiated wild running fits or wild running fits followed by tonic seizures 30 min. after the initial administration. PTZ doses (0.15-0.20 mg/kg ip) sufficient to effect observable behavioral response and EEG paroxysms reliably potentiated wild running fits followed by severe tonic-clonic seizures in 80% of animals tested. Time to initial seizure response following onset of the audiogenic stimulus was linearly dose dependent. In some animals a single TMPp administration potentiated an audiogenic seizure response when animals were acoustically stimulated up to three months later. PTZ (0.0-25.0 mg/kg ip) potentiated audiogenic seizures in naive adult Sprague-Dawley rats 30 min. following the initial dose. This result was unexpected, and may reflect the use of a mixed frequency (8,000 Hz predominant frequency, 110 dB for 60 sec.) audogenic stimulus as opposed to the traditionally used bell or pure tone. The results are discussed in terms of possible neurological mechanisms of action.

593.2

DIFFERENTIAL SUSCEPTIBILITY OF BALB/c, SWISS-WEISTER AND DBA/2 MICE TO INTRAHIPPOCAMPAL a-ACETYLENIC GABA. R. Urbanska*, G. Ceresaol, and R. Schwartz, Maryland Psych. Research Center, Baltimore, MD 21228.

Intrahippocampal application of the 'indirect' excitotoxin a-acetylenic gamma-aminobutyric acid (GABA) (240 nmol) caused a unilateral increase in seizures in rats (Exp. Neurol. 124:186, 1993). Unilateral GABA injections were made into the hippocampus of 3-week-old and adult Balb/c, Swiss-Webster and DBA/2 mice. Animals were killed 3 days later, and their brains were analyzed by light microscopy. Mortality, behavioral convulsions and hippocampal neurotoxicity differed substantially among strains (DBA/2 > Swiss > Balb/c). 360 nmol GABA had virtually no effect in Balb/c mice and led to seizure-related death in 30% of Swiss mice. In contrast, 90 nmol GABA caused 50% mortality in DBA/2 mice. Convulsions developed by 3-5 hrs post injection and often progressed to status epilepticus and tonic death. Hippocampal necrosis was seen in all (and only in) animals which had status epilepticus. Neuronal damage occurred bilaterally in area CA1 and often also in area CA3. Granule and hilar cells degenerated mostly ipsilaterally to the GAG infusion. In DBA/2 mice treated with 90 nmol GABA, the anticonvulsant valproic acid (2 x 350 mg/kg) and the NMDA antagonist CPP (40316 (20 mg/kg) totally abolished seizures and nerve cell death. In mice, therefore, GAG-induced hippocampal neurodegeneration seems to be seizure-related and mediated via NMDA receptors.

Supported by grant NS 16102.

593.3

ENHANCED KAINATE SENSITIVITY IN THE HAN-WISTAR RAT. B.W. Cohen*, C. Cepeda, C.A. Crawford, I.E. Margulies, L.B. Watson and M.S. Levine, Mental Retardation Res. Center, UCLA School of Medicine, Los Angeles, CA 90024.

We are studying a mutant Han-Wistar (HW) rat strain which displays a pattern of hippocampal degeneration (CA3 pyramidal cells) and mossy fiber sprouting similar to rat models of epilepsy (i.e., kainate-injected, kindling). We first studied the sensitivity of 40 day old mutants and littermate controls (n=20 pairs) to kainate injections (7-9mg/kg). Bipolar electrodes were implanted in the dorsal hippocampus and EEG recordings were made 3-5 days later. Prior to kainate injections, both mutants and controls did not show any abnormal paroxysmal discharges. After injection, behavioral and electrographic seizures occurred earlier (15-30 mins) and were more intense in the mutant. The mutants' seizures resembled the tonic-clonic type, while the controls exhibited short duration, high amplitude, paroxysmal discharges. Kainate injection was lethal to 50% (10/20) of the mutants but only 5% (1/20) of the controls. Another set of kainate-injected pairs (n=3) was analyzed by immunohistochemistry for the presence of c-fos, a gene regulating protein which becomes elevated in epileptic cells. Mutant hippocampus, which showed no differences c-fos expression prior to kainate treatment, exhibited a 30-40% increase in c-fos compared with controls. To determine a molecular basis of sensitivity, we examined the expression of non-NMDA glutamate receptor mRNA by in situ hybridization. We sampled all regions of the mutant hippocampus, suggesting an enhanced kainate sensitivity in the mutant. We propose that this strain, because of its kainate sensitivity, can be used to understand the complex relationship between cell degeneration and hippocampal reorganization in epilepsy.

593.4

PROCONVULSIVE ACTION OF PERIPHERAL-TYPE (OR MITOCHONDRIAL) BENZODIAZEPINE RECEPTORS IN ET AND 'NEURONAL' MUTANT MICE. Y. Nakamoto*, M. Yoshii, S. Watabe and T. Shitanai.

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We have recently reported that Ro 5-4864, a specific agonist for the peripheral-type (or mitochondrial) benzodiazepine receptor (PBR), can efficiently induce seizures in E1 mice, an animal model of epilepsy (Nakamoto et al., Soc. Neurosci. Abstr. 16, 380, 1992). To further characterize the proconvulsive action of PBR, we have examined the 'neuronal' mutant mouse, which develops a selective degeneration of cerebellar Purkinje cells with abnormal mitochondria. Ro 5-4864 at a dosage of 20 mg/kg or higher (i.p.) induced seizures consistently in nervous mice, whereas in their controls (heterozygous littermates) seizures could be induced at a lower dosage of 10 mg/kg. In binding assay for [3H]Ro 5-4864 in brain homogenates, the binding of [3H]Ro 5-4864 was reduced more than 50% in cerebella of nervous mice when compared with controls. In contrast, no significant changes in [3H]Ro 5-4864 binding were observed in forebrains between nervous and control mice. There was no apparent alteration in receptor affinity for [3H]Ro 5-4864. The results suggest that PBRs in the cerebellum could contribute to seizures as those in the forebrain.

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Parenteral administration of drugs can cause different rates of absorption. The glutamate agonist kainic acid (KA) is usually administered systemically by either the intraperitoneal (ip) or subcutaneous (sc) route to produce acute tonic limbic seizures in rats and is used as a model for temporal lobe epilepsy. In the present study, KA was systemically administered by ip, sc or intramuscular (im) routes for comparison. Fisher 344 rats were anaesthetized with a neutral pH by the ip, sc, or im route. Behavior was monitored for 30 minutes before and 240 minutes after injection of KA. The number of animals demonstrating behavioral seizures, number of animals exhibiting status epilepticus, behavioral seizure scores, latency to first seizure and latency to status epilepticus were compared for the three parenteral injection routes. Results showed trends for rats with sc injections to have shorter latencies to first seizure and to status epilepticus, for im injected rats to have the longest and ip injected rats intermediate latencies. There was also a trend for ip and im injected rats to have higher seizure scores than sc injected rats. However, there were no statistically significant differences between the three routes of parenteral administration on any of the seizure parameters measured. Seizure response variability due to route of administration will be discussed. Supported by the Department of Veterans Affairs.

THALAMIC-TRIGGERED SEIZURES. G.A. Cotrell*, H. Granasekaram and W.M. Burnham. Blooorview Epilepsy Laboratory, Dept. Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

Past research has shown that the midbrain and hindbrain can produce convulsive seizures when subjected to high levels of stimulation. The present experiment was designed to investigate convulsive behavior patterns elicited from the thalamus. Male Long-Evans rats were implanted with single bipolar electrodes using standard stereotaxic techniques. High-intensity electrical stimulation was administered to 14 thalamic nuclei, at 3 anterior-posterior planes. The posterior nuclei of the thalamus produced a generalized convulsion which was tonic or tonic-clonic, depending on the intensity of the stimulation. These convulsions resembled maximal electroshock seizures. Stimulation of the anterior and middle nuclei of the thalamus produced bilateral forelimb clonus, sometimes preceded by contralateral forelimb clonus. These seizures were never tonic, even at high stimulation levels. The data suggest that the thalamus is capable of producing convergent patterns, one purely clonic and the other tonic-clonic.

This research was supported by the Blooorview Epilepsy Program Grant and the Medical Research Council of Canada.

CITRULLINE INCREASE DURING TEMPORAL LOBEE SEIZURES SUPPORTS THE ROLE OF NITRIC OXIDE FORMATION IN EPILEPSY: AN IN-VIVO MICRORADIOISY STUDY. M.H. Shone, C.L. Wilson, N.P. Maina, E.H. Holgate, J.D. Roberts, J. Fried*. Deps. of Neurology, Anatomy, Psychiatry, Neurosurgery, and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Seizure initiation and spread involving glutamate (GLU) activation of the NMDA receptor may be mediated by Ca2+/calmodulin-dependent conversion of nitric oxide (NO) and citrulline (CIT) from arginine by nitric oxide synthase (NOS). Using in vivo microdialysis, Sorkin (Neuroreport, 1993, 4: 479-482) has shown an NMDA-evoked increase in the levels of CIT and GLU which is blocked by the NOS inhibitor L-NAME. Our hypothesis is that NO is involved in the cascade of events underlying seizure genesis in human epilepsy. Due to its gaseous nature, NO can diffuse to surrounding presynaptic terminals, increasing levels of cGMP and release of transmitter resulting in seizure genesis and propagation. In patients with intractable complex partial epilepsy who require diagnostic studies prior to temporal lobectomy, intracranial probes containing microelectrodes and microdialysis membranes were used to record EEG, single unit activity, and amino acid levels in the hippocampal formation, amygdala and entorhinal cortex. We have observed up to 5-fold increases in glutamate and citrulline during 3 out of 4 complex partial seizures in two patients, and a significant correlation in levels of glutamate with citrulline (r=0.71, p<0.001) both icatally and interictally. These increases provide evidence from the human temporal lobe in support of a role for NO in seizure genesis. Supported by NS 02808.

After global ischemia, rats tend to develop sound-triggered seizures which resemble those seen in genetically epilepsy-prone rats (Reid et al., FASEB J. 8:A660, 1994). We sought to maximize the fraction of post-ischemic seizures induced by the developed audiogenic seizures through an evaluation of 5 possible predictors of post-ischemic seizures in 60 rats. All rats received 7 minutes of chest compression under ketamine anesthesia at a skull temperature of 35°C. Resuscitated rats were tested for sound-induced seizures 24 hours later. The predictors evaluated were blood glucose, skull temperature, time to first spontaneous respiration, time to first EEG activity, and peak blood pressure during reperfusion. Receiver operating characteristic curves indicated that the best single predictor was the amplitude of isoelectric EEG; this had an accuracy value of 0.79. Combinations of predictors did not significantly improve this value, in part because we tried to hold all parameters constant so that the range of measured values was narrow. Supported in part by Alliant Community Trust Fund 9210.


Hippocampal CA1 region is known to be susceptible to global ischemia. The time-dependent change in this region of the post-ischemic seizure prone rats following chest compression was studied in 8 seizure prone rats and 2 controls. Rats were anesthetized and perfused fix 4 h, 48 h, 7 day, and 6 weeks following a 7 min global ischemia. Brains were removed, fixed for 3 more days, before being vibratom-sectioned at 40 μm for light microscopy or microdissected for electron microscopy processing. Decreased width of the stratum pyramidale and a concomitant increase in glial cell was observed in 7 day and 6 week samples. The early perivascular edema showed resolution through glial capping from day 7. Gradual neuronal death and glial migration as well as the presence of neurofilament-rich glia around the micro-vessels were verified by electron microscopy. These results confirmed selective vulnerability of the CA1 pyramidale cells and suggest that gliosis is an important sequela of this global ischemia model. Supported in part by Alliant Community Trust Fund 9210.


In this study we examined dynamic changes of monosynaptically evoked field potential in dentate gyrus (DG) by paired perforant path stimulus (25ms apart) for analysis of local GABA function during low-frequency (2Hz) DG kindling stimulus train using unanesthetized freely moving rats. We also observed extracellular field recording associating with afterdischarge (AD) triggering. During the kindling stimulus train, in all cases AD was triggered prior to occurrence of disinhibition of paired-pulse depression. Furthermore the triggered AD shapes changed with a population spike like component when the disinhibition occurred. By the kindling development, the paired-pulse depression was rather enhanced in the early period of stimulus train and latency until the disinhibition was prolonged. We consider that these findings suggest a possibility that collapse of local GABA mediated inhibition might contribute to seizure propagation rather than its initiation.


Following the recent theoretical prediction that chaotic physical systems might be readily controllable, there has been rapid and successful application of this technique to mechanical systems, electrical circuits, and even systems in cardiac tissue. One of the hallmarks of the human epileptic brain during periods of time in between seizures is the presence of aperiodic bursts of local synchronized neuronal activity known as interictal spikes. The high potassium in vitro hippocampal brain slice preparation exhibits population burst-firing activity that is in many ways analogous to the interictal spike. We sought to determine whether such neuronal bursting activity was amenable to control. Transverse slices 400 μm thick were prepared from the hippocampus of 125-150 gm female Sprague-Dawley rats with a tissue chopper, and placed in an interface type perfusion chamber at 32-35°C. Experiments consisted of combinations of slow control, periodic pacing, and the inverse of chaos control which we term anti-control. Ninety-one experimental trials were performed on 22 slices from 9 rats. Good control of this neuronal circuit was achieved in 14/52 chaos control trials, 8/19 trials using periodic pulses, and in 5/21 attempts at anti-control. This is the second attempt at achieving control of a chaotic biological system, and the first attempt in brain. Chaos control has the advantage over override periodic pacing in terms of its ability to identify and track the system's activity over time. In addition, the control of chaos during this experiment is possible by "anti-control". The anti-control method used here employs a minimum number of stimuli needed to prevent periodic behavior. Such techniques may be applicable to human epileptic foci.

Spike and wave high voltage spikes (HVS) pattern (7.8 Hz) in rodents is an animal model for petit mal epilepsy. These nonconvulsive generalized seizures originate in the thalamus and become bilaterally synchronous in the cerebral cortex through the neural interplay between thalamo-cortical and interictal networks. In this study, microdialysis probes were placed unilaterally to perfuse CNQX (0.01-1.0mM) and high-calcium ACSF (2.4-30.0mM) into the ventral basal (VAVL) and reticular (VTN) thalamic nuclei to alter seizure activity in awake nonanesthetized freely-moving rats. This form of drug administration greatly reduces repeated handling, which can affect seizure rate, while enabling the infusion of dialysates into discrete brain regions. EEG was recorded, after 1 hour of drug washout, for 2 hours with epidural screw electrodes.

High-voltage concentrations did not change overall HVS duration but seizures were limited to the time of calcium perfusion and greatly reduced during normal ACSF perfusion. Calcium infusion outside of the VAVL, VTN did not exhibit this effect. CNQX perfusion: 0.01mM created a 2 Hz delta, 0.1mM amplitude spiking pattern ipsilateral to the probe while not affecting the contralateral HVS pattern. 0.1mM induced a 2 Hz, 0.2mM ipsilateral spiking pattern, while suppressing the contralateral HSV or created ipsilateral 1 mV spiking without affecting the contralateral HSV or decreases ipsilateral HVS. 1.0mM suppressed HVS bilaterally but not completely contralateral to infusion. In most cases HVS durations and amplitudes recovered after 24 hours of ACSF infusion.

These experiments further underline the importance of calcium and the AMPA glutamatergic system in the thalamic oscillations underlying petit mal epilepsy.


Department of Anatomy, California State University, Fullerton, CA 92634. 1. The kainate-treated rat, an animal model of temporal-lobe epilepsy, undergoes an initial period of status epilepticus that can ultimately result in a permanent epileptic state. The aim of the present experiments was to determine the initial effects of kainate-induced seizures on evoked field potentials from dentate granule cells in freely-behaving rats. Chronic in vivo recordings were performed during a 2 day period prior and 1 day after kainate treatment. Rats (n=3) were given pentylenetetrazol (PTZ; 2 mg/kg per hr) for 4-8 hr, and class IV seizures were elicited for ≥ 3 hr. Prior to kainate treatment, single stimuli of the perforant path (at maximal intensity) evoked a positive field-potential PSP that lasted for 15-20 ms with one to two superimposed population spikes. Paired-pulse stimulation of the perforant path (interpulse interval = 20 ms; stimulus intensity that produced a half-maximal population spike) showed attenuation or little change of the field PSP and population spikes to the second stimulus. One day after kainate-induced status epilepticus, however, single stimuli evoked prolonged field PSPs (duration = 63-75 ms) with multiple population spikes. Paired-pulse stimulation led to facilitation of the field PSP and population spike to the second stimulus. The prolongation of the field PSP and the paired-pulse facilitation suggests that recurrent inhibition was depressed in the dentate gyrus of the kainate treated rat. Although further experiments are necessary, these results suggest that relative rapid changes in dentate electrophysiology, apparently including recurrent inhibition, occur following a period of kainate-induced status epilepticus. Supported by NIH grants NS16683 and NS28893.

594.10 MEMBRANE PROPERTIES OF CA3 HIPPOCAMPAL REGION IN THE GENETICALLY EPILEPSY PRONE RATS. Suntaneta Verma-Abuji, M. Steven Evans, and Teresa L. Fenecke, Department of Surgery, Division of Neurosurgery, and Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, Illinois, 62294-9200.

Genetically epilepsy-prone rats (EPR) exhibit a generalized increase in seizure susceptibility. Several factors indicating an increased excitability in CA3 region of the EPRs have previously been reported. These included an increased input resistance, reduction in spike frequency adaptation, less current required to elicit an orthodromic EPSP and marked facilitation with repetitive stimulation in CA3 region. We now have recorded membrane properties from 15 neurons in the CA3 region of Sprague Dawley (SD) rats and five neurons in the EPRs CA3 region. The resting membrane potential and action potential amplitude were not different between the two. A significant difference was seen in the spike frequency adaptation in the GEPR neurons. In SD neurons a suprathreshold stimulus of 800 μs elicits a burst of action potentials followed by a prolonged hyperpolarization lasting 600 to 750 ms. No action potentials were observed following the burst in these neurons. In GEPR neurons there was a significant increase in the number of spikes in the burst. This increased firing in the burst was not followed by a hyperpolarization, and action potentials were elicited throughout the 800 ms depolarizing stimulus. This suggests an abnormality in the calcium or calcium dependent potassium conductance.


Exposing immature rats to hypoxia evokes epileptiform activity which is dependent upon both age and oxygen concentration (Ann. Neurol. 20:629-637). Although perinatal hypoxia leads to increased long term seizure susceptibility, morphological damage has not been demonstrated (Epilepsia 33:971-980; Life Sci. 31:1597-1604). We have attempted to identify hypoxic conditions which will produce short-term hippocampal damage in order to study the possible functional abnormalities resulting from such trauma.

Rats representing 3 age groups (P8-P12, P15-P17, and P20-P27) were exposed to either 15 minutes of anoxia or 3 successive 5 minute single 60 minute treatments. Silver degeneration and Nissl staining revealed no difference between treated and control animals at 1, 4, or 7 days following treatment. Fifteen minute hypoxic exposures (or P8-P12 or P9-P11) did not significantly affect CA1 field potentials or intrinsic properties, but may have altered fast IPSP conductance in hippocampal slices prepared 1, 4, or 7 days after the third treatment.

The immediate posthypoxic period in the hippocampus is characterized by a combination of hypoxia and ischemia. We found that, whereas neither hypoxia nor unilateral carotid ligation alone produced neuropathology, the combination of the two reliably produced neuronal damage (see also Ann. Neurol. 13:131-144). In P8-P12 rats, unilateral carotid ligation followed by 60 minutes of hypoxia induced light terminal degeneration in the terminal field of the mossy fiber (the stratum lucidum in CA3) as well as apparent transcellular cell damage in the pyramidal cell region. Supported by NIH, NINDS grant NS 15117.

594.12 CORTICAL MICROGLIA ARE EPILEPTOGENIC IN VITRO. K.M. Jacobs*, M.J. Gurnick, and D.A. Prince. Dept. Neurology & Neurological Sciences, Stanford University Medical Center, Stanford, CA 94305.

Cortical microglia, thought to be the result of abnormal neuronal migration, are commonly found in the brains of epileptic patients. Clinical data suggest that seizures in some form of malformation of cortical development (MCD) are linked to the presence of bilateral structural abnormality, although mechanisms underlying epileptogenesis in the maldeveloped cortex are unknown. We used a method preparing experimental microglia in rats described by Dvorak and Feit (1977), in which an intracerebral injection of Trypan blue in the 4th ventricle stained the microglia. In initial experiments, Trypan blue microglia were found in the cerebral cortex only in rats treated with a combination of hypoxia and ischemia. We found that, whereas neither hypoxia nor unilateral carotid ligation alone produced neuropathology, the combination of the two reliably produced neuronal damage (see also Ann. Neurol. 13:131-144). In P8-P12 rats, unilateral carotid ligation followed by 60 minutes of hypoxia induced light terminal degeneration in the terminal field of the mossy fibers (the stratum lucidum in CA3) as well as apparent transcellular cell damage in the pyramidal cell region. Supported by NIH grants NS04677, NS12151 and NS07288.

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It is still a question of much debate whether single epileptic seizures can cause cell loss. A critical implication that epilepsy in general is a progressive disorder, experimental evidence is not conclusive on this point. Recently, it has been shown electrically-induced afterdischarges of less than 2 minutes may induce structural impairments in neurons (Soc. Neurosci. Abst. 1992, 18:553). Here we evaluated whether spontaneous seizures could lead to structural changes. Chronic spontaneous recurrent seizures were induced with pilocarpine (320 mg/kg, i.p.). Animals were sacrificed from 1 h to 6 h after single or multiple seizures. A Golgi-like sensitivity and silver-staining procedure (J.Comp.Neural. 1990, 298:654-673) was used to reveal injured neurons. Silver-stained dark neurons were never found in control animals or in epileptic animals that had no behavioral seizures in the 8 h prior to sacrifice. After spontaneous seizures (injured) dark neurons were mostly interneurons and were present in hippocampus (CA I stratum radiatum), amygdala, piriform cortex and other limbic structures. Animals with multiple seizures had a higher number of dark cells than animals with single seizures. Our findings suggest that even single generalized spontaneous tonic-clonic seizures can induce long-lasting morphological changes. Our results favor the idea that epilepsy is a progressive disorder in which 1 seizure begets the next. Supported by: FAPESP, CNPq and FINEP, L.C. is a CNPq fellow.

AXONAL ARBORIZATIONS OF BICYTIN FILLED CA1 PYRAMIDAL CELLS FROM HYPEREXCITABLE SLICES OF KAINATE TREATED RATS. Y. Perez, F. Morin, J. Jatras, C. Beaulieu and J-C. Lacaille. Center for Research in Neurobiology and Department of Physiology and Pathology, University of Montreal, Montréal, QC, Canada H3T 3J7.

Following hippocampal kainic acid (KA) lesions, CA1 pyramidal cells become hyperexcitable. To examine if sprouting of CA1 pyramidal cells contribute to this epileptiform activity, the axonal arborizations of intracellularly-marked pyramidal cells have been compared in control and hyperexcitable slices of KA treated rats. Hippocampal slices were obtained from Sprague-Dawley rats 2-4 weeks after bilateral intraventricular injection of KA (0.65 µg). Hyperexcitable slices were identified using cholera toxin B subunit (CTB) retrograde labeling. CA1 cells were impaled in control (uninjured) or hyperexcitable slices with microelectrodes containing bicytin (1%) in 1M K-acetate. Synaptic responses were recorded at 100 Hz. Axons from pyramidal cells that could be followed from the soma to the axon were drawn with a camera lucida. In cells from CA1 of control slices, axon branches on average once (mean branch points 0.9 ± 1.0) before entering the alveus and then coursing toward the fimbria or subiculum. In CA1 cells from KA lesioned CA1 pyramidal cells develop more extensive local axon collaterals and this sprouting may contribute to the epileptiform activity.

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Long-term changes in animal behavior and hippocampal tissue were investigated on the male Wistar rats in which limbic seizures were induced at the age of 5 weeks by subcutaneous injection of kainic acid in order to clarify any plastic changes following seizures. The rats were injected with 50 µg/100 µl of kainic acid at 3-5 weekly intervals until 1-3 hours by diazepam, neuronal loss was observed in the CA1 pyramidal layer of the dorsal hippocampus. Morris water maze learning with a hidden platform) and passive avoidance learning were impaired within two weeks after the seizure. However, the rats learned these tasks well when tested 1-2 months later. A small number of neurons survived in the lesioned pyramidal layer and extended their dendritic branching obliquely and tortuously. Synaptophysin/SV2P immunostaining and cholineresterase histochemistry were not changed in the lesioned CA1, suggesting that some synapses and axon fibers were well maintained. Astrocytes proliferated there and some of them immunoreacted on anti-GABA and anti-glutamate decarboxylase (GAD) antibodies. On the other hand, when the seizures continued over 5 hours without diazepam, the lesion was more extensive and permanent. Neuronal loss was observed in CA1 and the subiculum and the hippocampus was atrophied. The rats did not recover from the impaired water maze learning.


Injection of 75 µg aminoxyacetic acid (AOAA) into the rat entorhinal cortex (EC) produces acute behavioral seizures and selective neuronal loss in layer III of the region (Neurosci. Lett., 147:185, 1992). Since the EC provides a major efferent input to the hippocampus, we examined the hippocampus of rats receiving an entorhinal AOAA injection using Nissl staining and heat shock protein (HSP) immunohistochemistry. BSEP expression has been considered a specific marker of excitation-induced neuronal stress. Rats were sacrificed after 15 or 24 hours, or 5 days. After 5 days, neurodegeneration and gliosis were readily observed in Nissl-stained sections in the subiculum and CA1 field of the ventral hippocampus ipsilateral to the injection site. WSP immunoreactivity (-) were detected slightly after 15 hours following the injection, mainly in the subiculum, CA1 and in the hilus. Rats of HSP-1 neurons remained similar at 24 hours and 5 days after AOAA treatment. These results indicate that injection of AOAA into the EC produces a long-lasting degeneration of postynaptic neurons in the rat hippocampus. AOAA injection into the EC in rats may therefore provide a novel model for temporal lobe epilepsy. Supported in part by USPHS grant NS16102.
595.7

THE DENSITY OF DENTATE GRANULE CELL SPROUTING IN HEAT-INDUCED SEIZURES IN THE RAT IS RELATED TO THE NUMBERS OF SEIZURES SURVIVING THE FIRST 14 DAYS. J.H. Kang, B.D. Korfine, and J.C. Lendale*. Section of Neuropsychology, Yale Univ. School of Medicine, New Haven, CT. 06510.

The sprouting of mossy fiber collaterals into the inner molecular layer (IML) of the dentate gyrus has been observed in patients with temporal lobe epilepsy (TLE) (Brain Res, 189, 493-587). Patients that show this phenomenon are often found to have a history of febrile seizures. The relationship of the degree of sprouting to seizure history remains speculative in humans because of the difficulty of obtaining accurate information on the subject. Rat pups experiencing heat-induced seizures also show sprouting. These seizures are ionic-toxic seizures (lasting 30 sec to 5 min) and not status epilepticus, thus resembling seizures in TLE patients. The relationship of the degree of sprouting to the duration of seizures was evaluated in this experimental model. Variable numbers of seizures (1, 6, 12, 24) were induced in different experimental groups of rat pups beginning at 22 days of age. Seizure induction was by exposure of a rat for 4 minutes to water at 45°C. Consequent seizures were induced every fourth day in the multiple seizure groups. The core body temperature of the rats rose from approximately 38°C to 44°C on exposure. Sprouting was observed by the Timm stain. No sprouting was found in the IML of the dentate in the one seizure group. In the six seizure group Timm staining positive particles were limited to the tips and angle of the dentate blades in the IML. Sprouting into the IML was seen in the 12 seizure group, sprouting being strongest at the tips and angle of the blade than in the six seizure group. In the 24 seizure group there was considerably increased sprouting which extended in the IML throughout the entire of the dentate gyrus, with most intense stain at the tips and dentate blade angle. These results support the hypothesis that sprouting is the result of the seizures, and more seizures produce more sprouting. Supported by NS 06038.

595.8

CORTICAL CONOTOXIN BINDING DIFFERENTIATES EOSPHAGAL AND NON-EOSPHAGAL MICE. S.K. Jensen, A.F. Burowolls, F. Matsuo, and M.J. Litzinger. Laboratory of Applied Neurology, Department of Pediatrics and *Neurology, University of Utah, Salt Lake City, UT 84132.

Espelin et al. (Epilepsia, 1994) have suggested that N-type presynaptic voltage sensitive calcium channels (VSCC) are differentially expressed in epileptic (DBA/2J) and non-epileptic (C57/B1) mice. Binding with the presynaptic calcium channel probe α-conotoxin, believed to mark N-type voltage sensitive calcium channel (VSCC), was demonstrated in the cerebellum but no differences in synapse formation between these two mouse types. Whole brain α-conotoxin binding was measured by the eye opening postnatal day (PND) 6 C57/B1 mouse brain to have fewer binding sites than its epileptic counterpart DBA/2J mouse. Postnatal day (PND) 16 C57 mouse have significantly more binding sites than the DBA mouse.

The purpose of this study was to show that the majority of binding in the whole brain studies came from cortical VSCC development. Regional dissections were performed as previously described (Litzinger et al., J. Child Neurology, 1994). Preliminary data indicates that the cortex is responsible for the developmental binding differences seen in the whole brain preparations from these two different mouse types. Cerebellar and diencephal-brainstem binding remains relatively unchanged. This data suggests that pre-synaptic VSCC's in the cortex are potentially involved to the seizure susceptibility described by Espelin. For discussion of functional differences see Burrows and Litzinger, this section.

595.9


Induction of seizure activity utilizing a variety of techniques including electroconvulsive shock (ECS) and amygdaloid kindling has consistently been reported to produce alterations in thyrotropin releasing hormone (TRH) concentration and increases in prepro-TRH mRNA expression in discrete brain regions. Studies in our laboratory have shown that induction of seizures by kainic acid administration, which activates limbic pathways preferentially, has a more pronounced and prolonged effect on limbic TRH concentrations. To further elucidate the neurochemical basis of the effects of seizure activity on TRH neuronal systems, we have utilized hybridization methods to study systemic kainic acid administration on the expression of prepro-TRH mRNA in limbic brain regions. Male Sprague Dawley rats (180-200g) received a single intraperitoneal injection of either kainic acid, 12mg/kg, in 0.9% NaCl, or vehicle. Separate groups of rats (n=4) were sacrificed by decapitation at 6, 24, 48, and 72 hours, and 14 days post-seizure. Rat brains were dissected at 10cm and processed for in situ hybridization using a [35S]-labeled riboprobe to detect TRH preprohormone mRNA. Significant increases in preproTRH mRNA at 6 and 24 hours were observed in the hippocampus, amygdala, piriform cortex, entorhinal cortex, and the reticular thalamic nucleus following a single stage 5 seizure. In the hippocampus, prepro-TRH mRNA grains were most dense in the dentate gyrus region. After reaching a peak within 24 hours, message levels in these areas gradually declined and reached baselines by 14 days. These findings provide further support for a role of TRH neuronal systems in seizure modulation.

595.11


Employing Northern blot analysis, we examined in the rat the postnatal development of c-fos mRNA expression induced by systemic administration of pentylene trinitrate (PTZ)(50mg/kg) induced after a hour a high level of c-fos mRNA in the neocortex at 23 and 49 days, but not at 8 and 14 days, postpartum as compared with the saline-injected control animals. The induction increased gradually from day 23 to 49, and declined dramatically from day 23 to 49. In contrast, c-fos mRNA was nearly undetectable in the hippocampus of both groups throughout day 8 to 49. Induction of the proto-oncogene c-fos is considered to be a marker of neuronal activity. The present results thus indicate that neocortical neuronal circuits responsive to the drug may alter remarkably between day 23 and 49.

595.10

LONG TERM UP-REGULATION OF OXYTOCIN MESSENGER RNA EXPRESSION IN RAT PVN FOLLOWING KAINIC ACID INDUCED SEIZURES. Q.Sun, S.Peter*, C.D. Applegate, D.Pautom, Departments of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642.

The paraventricular nucleus of hypothalamus (PVN) is the neuroendocrine center for stress-related responses. It is known that the CRF neurons of the PVN have an important role in regulating stress responses of the CNS. Vasopressin and oxytocin (OX) neurons of PVN are traditionally associated with the regulation of CNS responses to disturbances of cardiovascular. However, these neurons might also be involved in CNS responses to other stressful stimuli. Our laboratory had previously shown that the Fos antibody was expressed in OX neurons of PVN in both electrical and kainic acid induced seizures, indicating these neurons were activated by the stress stimulation induced by seizures. To further characterize the involvement of OX in response to seizures, OX mRNA in rat PVN was examined with quantitative in situ hybridization in kainic acid induced seizure. No OX mRNA was observed in PVN seizure animals more than 20% higher than that of control animals at all time points of survival. The level of OX mRNA occurred as early as 1.5 hour following the first stage 5 seizure and remained elevated as long as 4 weeks. These results demonstrate that OX neurons of PVN respond to seizure by up-regulating their mRNA expression. They also demonstrate that this response represents a long term effect of seizures on magnocellular PVN neurons. Supported by NIH grant NS 18626.

595.12

NEUROANATOMICAL CONCORDANCE OF ABNORMAL TYROSINE HYDROXYLASE AND c-fos mRNA EXPRESSION DURING FOCAL MYOCYCLIC EPISODES IN THE MOUSE MUTANT TOTTERING (tptg). L.J. Hess* and M.L. Williams, Departments of Neurosceience & Anatomy, The Pennsylvania State University College of Medicine, Hershey Medical Center, Hershey, PA 17033.

Tottering (tg) is a labile autosomal recessive mutation in the mouse that results in a trait of neurobehaviours including spike and wave discharges, ataxia and focal myoclonus. The spike and wave discharges and ataxia have been extensively characterized, however the myoclonus expressed by these mice has been difficult to study because these seizures have no obvious electrical correlates. We have previously identified the myoclonus as a subcortical phenomenon occurring in thalamo-pontine neurons using the expression of the immediate early gene (IEG) c-jun to chart the progression of abnormal nervous system activity. In situ hybridization revealed myoclonus-induced c-fos mRNA expression only in the cerebral cortex and brainstem nuclei and faintly in motor cortex with the highest levels of expression in the cerebellum. We have refined these results and have found that c-jun expression in the cerebellum occurs in the more medial aspects of the cerebellum and is not induced in Crus I or Crus II. Thus, these seizures appear to delimit functionally distinct regions of the cerebellum. We have also previously observed a substantial reduction in basal c-fos expression in brain stem and compared the time course of expression of tyrosine hydroxylase (TH) in the Purkinje cells of these mutants (Neuron, 6:125). Interestingly, TH expression is also restricted to the medial regions of the cerebellum. In fact, in situ hybridization on back to back sections of the cerebellum revealed that c-jun induction during a myoclonic episode occurs in granule cells adjacent to Purkinje cells abnormally expressing TH. These results suggest that not only is the cerebellum important in the generation and maintenance of myoclonus, but it appears that very specific cerebellar regions are affected by the mutation. Additionally, these results suggest that c-jun may directly contribute to the phenotypic expression of the mutant mutation. Supported by a Klingenstein Fellowship.
595.15 GABA<sub>A</sub> RECEPTOR FUNCTION IN THE THALAMUS AND CORTEX IS ALTERED IN RATS WITH EXPERIMENTAL ABSENCE-LIKE SEIZURES. G. Czerneck, M. A. Ivey and P. K. Reuter. Div. Neurol. Childrens Hospital of Los Angeles, Dept. Neurol. Univ. Southern California, School of Medicine, Los Angeles, CA 90027

Both clinical and experimental absence seizures are exacerbated by GABA<sub>A</sub> agonists. We investigated GABA<sub>A</sub> receptor activity during the course of absence-like seizures induced by 4-hydroxybutyric acid (GHB) in rats using receptor autoradiography in brain sections obtained at different time intervals during GHB-induced seizure activity. The 3H-flunitrazepam and [35S]TPPS<sub>3</sub> binding in the parietal cortex was increased, and in the temporal cortex, both binding sites were decreased and increased, respectively. The 3H-flunitrazepam binding was also increased in the thalamus (approx. 25-35%) and cortex (approx. 10-15%) associated with a brief loss of ALP-induced increase in [35S]TPPS<sub>3</sub> binding observed which returned to baseline levels 10 min after the onset of seizures. 3H-flunitrazepam binding rose in midline thalamic structures (by 10%) and cortex (by 15%) throughout GHB-induced absence seizures. There was a selective loss of ALP-induced enhancement of [35S]TPPS<sub>3</sub> binding in cortex, but not thalamus during the course of seizure activity. These results suggest that GABA<sub>A</sub> receptor activity is modulated in a subtle fashion during experimental absence seizures induced by GHB. Whether this is the cause or effect of the experimental absence seizures is not clear.


Sprouting of the mossy fibers into the supragranular region of the dentate gyrus is a well-known regenerative process after epileptic injury. No consensus has been achieved so far about whether new circuitries form as a result of sprouting are excitatory or inhibitory in nature. This study investigated an uncharacterized target for sprouted mossy fibers using a combination of immunofluorescent and Timm stainings. Two months after the injection of mossy fiber sprouting by kainic acid injection, rats were perfused transcardially with 4% paraformaldehyde. Immunolabeled parvalbumin and calbindin were first immunostained for parvalbumin (PV) or calbindin D-28k (CaBP) using FITC-labeled antibodies. Then they were stained using Timm sulfide-silver histochemical method. Analysis of parvalbumin-positive and calbindin-positive neurons and processes was made using the confocal laser scanning microscope revealing that the Timm-positive mossy fibers were in close contacts with the PV- and CaBP-immunopositive neurons within the granule cell layer, through which the sprouted mossy fibers were spreading in the kainic acid injected rats. These results suggest that the PV- and CaBP-containing cells are the targets for the sprouted mossy fibers. Therefore if PV is present in the inhibitory nonpyramidal cells and CaBP in the granule cells sprouting results in formation of both inhibitory and excitatory circuitry. However, it should be noted that we cannot rule out the possibility that the CaBP-positive neurons which were found to be surrounded by mossy fibers in the granule cell layer are not CaBP-containing nonpyramidal cells, and thus inhibitory in nature.
596.3 ANTECEDENT TONE PRESENTATION SIGNIFICANTLY DELAYS RATE OF AMYGDALA KINDLING T.D. Hernandez*, I.A. Warner, P.J. Kahler and A.E. Klugs, Department of Psychology, University of Colorado, Boulder, CO 80309

The degree to which external stimuli contribute to seizure occurrence is an important issue. Can external events elicit the aberrant neuronal activity that precipitates a seizure? Alternatively, can certain external events cue the organism to an impending seizure and result in a compensatory response that is in effect "anti-convulsant"? While several studies have addressed these issues (Hu & Yamaguchi, 1963; Finet et al., 1973; Wyler & Hevner, 1979; Jarowsky et al., 1980; Mostolosky & Myloobdsky, 1982; Myloobdsky et al., 1983; Freeman R.B. Kindling, 1986) the results have been inconsistent. Consequently, we conducted the present study in an attempt to clarify these issues. Twenty-seven male Long-Evans hooded rats were chronically implanted with electrodes in the right amygdala and randomly assigned to one of two groups. The Tone group was presented with a 2-second tone beginning immediately prior to and overlapping with the daily kindling stimulus, while the No Tone group was kindled without the tone. Presentation of the tone significantly delayed the rate of amygandal kindling. All animals in the No Tone group reached a Stage 5 kindled seizure by the 12th daily stimulation, while all animals in the Tone group responded with a Stage 5 seizure by the 22nd daily stimulation. These data indicate that the antecedent presentation of a mild auditory cue has a profound inhibitory effect on the rate of kindling.

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596.5 HYPO- or HYPERTHYROIDISM DOES NOT AFFECT AMYGDALA KINDLING-INDUCED THYROTROPIN-RELEASING HORMONE (TRH) mRNA INCREASES IN LIMBIC STRUCTURES. S.L. Kim* and J.B. Rosen, Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

Our laboratory has demonstrated that TRH mRNA is dramatically increased following amygdala kindling in limbic structures including dentate gyrus, and pyriform, entorhinal and perirhinal cortices, but not in the paraventricular nucleus of the hypothalamus(PVN). The regulatory mechanisms of kindling-induced TRH mRNA expression are not known. Since thyroid hormone regulates TRH mRNA in the PVN, we investigated whether kindling-induced TRH mRNA elevations in limbic regions are also regulated by thyroid hormones. Rats were treated with 0.05% propylthiouracil (PTU) (equivalent to ~30 mg/kg/day) or 4 ug/ml triiodothyronine(T3) (equivalent to ~500 mcg/kg/day) in drinking water for 10 days before kindling and throughout the kindling protocol. Rats were sacrificed 4 hours after a fully kindled seizure. The efficacy of oral treatment of PTU and T3 was confirmed by Northern blotting hybridization of pituitary thyroid-stimulating hormone(TSH) mRNA. TSH mRNA was significantly increased by PTU and decreased by T3, but unaffected by kindling. In addition, in situ hybridization showed that PTU increased and T3 decreased TRH mRNA in the PVN while kindling had no effect on TRH mRNA in the PVN. In contrast, kindling significantly increased TRH mRNA in dentate gyrus, pyriform, entorhinal and perirhinal cortices regardless of PTU or T3 treatment. The findings demonstrate that unlike its effects on hypothalamic TRH mRNA, thyroid hormone manipulation does not alter baseline TRH mRNA expression in extrahypothalamic areas and does not affect TRH mRNA expression following amygdala kindling. These data suggest that hypothalamic TRH mRNA and seizure-induced TRH mRNA in limbic structures are regulated by different transcriptional mechanisms.

596.6 EXTENDED KINDLING RESULTS IN SPATIAL LEARNING DEFICITS IN THE RAT. J.Th. Rick*, S. Cammeule, C.J. Reid, M.P. Murphy, M. Michaela, J. Farbintaneu, and N.W. Milgram, University of Toronto, Scarborough Campus, 1265 Military Trail, Division of Life Sciences, Scarborough, ON, Canada M1C 1A4.

Kindling is a model of epilepsy in which repeated electrical stimulation of various forebrain structures leads to the progressive development of motor seizures. Impaired spatial cognitive functioning, sometimes seen in epileptic patients, has not been observed in kindled animals except when tested shortly after a seizure. Previous kindling studies have reported no long-term deficits in spatial learning, but stimulation was terminated after fewer than 10 class 5 seizures (Baceline Scale). Moreover, more seizures may be required before cognitive deficits arise. In the present study, a total of 12 male Long-Evans hooded rats were stimulated 1-3 times daily through electrodes implanted either in the amygdala (N=5) or perforant path (N=7) until they became spontaneously epileptic or received 300 stimulations. Twelve additional animals with matched electrode placements served as controls. Kindled rats experienced up to 290 class 4 or greater seizures and 5 of these subjects experienced spontaneous motor seizures. Ten days after the termination of kindling, the rats were given 10 trials per day for 3 days in the Morris water maze. Kindled rats required more trials to reach the learning criterion (3 consecutive trials with escape latencies under 20 s) than controls (p<0.0003). The number of major seizures (class 4 and up) was a significant predictor of poor performance (p<0.003). These results suggest that extended kindling can lead to a persistent spatial memory deficit in the rat.


The hippocampus has long been known to play an important role in spatial learning, that interference with hippocampal functioning can produce deficits in tasks that involve spatial learning. Leung (1989) reported that kindling of hippocampal field CA1 disrupts performance in the radial arm maze and that this disruption was caused primarily by hippocampal afterdischarges (ADs) in the absence of behavioral seizures. We asked whether CA1 kindling would disrupt spatial learning in the Morris water maze.

We used two procedures: (1) seizures were kindled with stimulation of CA1 prior to training in the maze (acquisition); and (2) seizures were kindled following maze training, and then retesting in the maze occurred after kindling (retention). In both cases, experimental rats received one daily CA1 stimulation until 3 consecutive generalized seizures were evoked, or until a predetermined number of ADs was reached without eliciting generalized seizures (p<0.05). The rats were tested (or retested) in the maze 24 hours after the last seizure or last AD.

We found that CA1 kindling failed to disrupt maze performance during either acquisition or retention for both rats kindled partially and fully kindled groups, which demonstrated acquisition and retention performance comparable to that in nonkindled controls. These findings are inconsistent with previous results suggesting that memory deficits occur during or after hippocampal kindling and may indicate that deficits are task-specific. (Supported by NSERC)


A single topical dose of Penicillin (Pm) induces a dose dependent "massed" amygdaloid kindling. Interictal spikes (1-3 cps) appear in 1 or 2 minutes after Pm administration and last for several hours. 20 Wistar male rats were anesthetized when used. A calcium-electrode was stereotaxically implanted into the medial amygdala, and a bipolar electrode was placed in the right amygdala. For cortical recording and mapping, a 16 epidural monopolar electrode (stainless steel) array was implanted covering the whole dorsal cortex (frontal, Rolandic, temporo-parietal and visual). Mapping of restricted cortical areas was performed with a moveable 4x4 electrode matrix covering 16 mm².

After a 40 min control recording, each rat was given a dose of Penicillin ranging from 50 to 200 IIU/1 ml which were microinjected in the base-lateral amygdaloid nucleus. Naloxone was IP delivered (0.5-1.0 mg/kg) after the injections. A/P Activity in the NAc and Se was compared before and after naloxone (10 µg/kg). Amygdaloid Penicillin bsp spikes with a variable latency and progressive enhanced frequency and amplitude were observed during a period of 35 min. After a plateaus (1-3 Hz), a frequency decrease was observed. Spiking frequency and amplitude diminished in about 45 min. (200 IUJ). Some animals displayed brief (5 to 10 sec) focal or generalized seizures during this period. Naloxone increased the interictal spike frequency and amplitude. Finally, both massed and massed inhibited Pm spiking when delivered after Pm. When delivered before Pm, the enkephalins depressed the interictal Pm spiking or avoided the Pm effect.

We conclude that enkephalins elicits focal discharges and that this inhibition is blocked by Naloxone.

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596.9 THE TIMING OF PERMANENT ALTERATIONS IN SEIZURE SUSCEPTIBILITY DURING KINDLING. J.L. Burdeett* and C.D. Applegate. Comprehensive Epilepsy Program, Dept. of Neurology, Univ. of Rochester School of Medicine, Rochester, NY 14642.

When does kindled seizure development become permanent? It is well known that kindling is permanent after stage 5, but are permanent changes induced at earlier stages. Specifically, does each successive afterdischarge induce permanent alterations through the kindling process or does the process become permanent only after some threshold is reached. We designed an experiment to choose between these two possibilities.

Adult Sprague Dawley rats were kindled from the septal nucleus. Different groups of animals received 6, 9, 12, 15, or 21 stimulations, respectively. Then the animals were stimulated for 4 weeks and the histology, activities and seizures were again stimulated until they reached stage 5 seizures.

If each afterdischarge induces a permanent increase of seizure susceptibility, then the bimodal pattern of development should affect subsequent kindling development. We would predict that: (1) the total number of trials to reach stage 5 should be the same for each group, and (2) the number of post-lusists trials should be invariant related to the number of trials for each group.

The outcome was inconsistent with these predictions, suggesting a lack of permanent alteration of seizure susceptibility during early kindling trials. We conclude that there is a threshold for permanent changes during kindled seizures development. Our data are most consistent with this threshold occurring during the transition from stage 2 to stage 3.

Supported by NID grant NS20351.

596.10 LATERAL OLFACTORY TRACT IS THE OPTIMAL SITE FOR KINDLING T.J. Neto(e), W. Freeman(a), F. Margalh(+) and M.B. Diogo(-). (+) MC-B -Neuroscience. (-) School of Optometry, University of California at Berkeley, Berkeley, CA 94720.

In the late 1960s Graham Goddard discovered the phenomena of kindling, and that stimulating the amygdala produced kindling the fastest. For years this result was not easily replicated. Recently we have discovered that stimulation in the deep layers of the pyriform cortex (PC) caused kindling to occur even more rapidly. This lead us to look at the lateral olfactory tract (LOT) as a stimulus site. We have found that when kindling here requires fewer seizure sessions to bring an animal to stage 5 kindling than stimulating in the amygdala. The olfactory bulb (OB) was found to have the background excitation for the PC through the LOT. Stimulation of the LOT is more efficient than directly stimulating the PC in two ways: 1) the stimulus in the LOT projects broadly to the PC stimulating a large portion of the PC directly and not just a small area around the electrode; 2) the stimulus in the LOT stimulates the OB antidromically as well, thus altering the dynamics of the limbic system very efficiently. The other change we have made from the standard kindling model is the stimulus duration and intensity. The stimulus is much milder (0.1-0.2 ms duration, 60 Hz, 10-15 V) and impedance has been matched to the LOT so that the largest evoked potential in the OB and PC are observed. However, the stimulus requires a longer train (60-10 sec) to elicit a seizure.

596.11 THE KINDLED STATE, BRAIN DAMAGE AND SEIZURE SUSCEPTIBILITY. Craig D. Appleget* and Gary M. Samoriski. Comprehensive Epilepsy Program and Dept. of Neurology, University of Rochester School of Medicine, Rochester, NY 14642.

Several studies suggest that the kindled, kindling-induced increase in seizure susceptibility is not static, but continues to evolve over time. Our studies support this interpretation, and further indicate that impairment of the body's ability to chronically indwelling electrodes significantly contribute to increased seizure susceptibility. Male, C57BL mice were stereotaxically implanted with electrodes into the olfactory bulb and following a 4 week recovery were kindled to a criterion of 6 consecutive stage 5 seizures. Seizure threshold to fluorode (HFE) was tested and 20 days following the last seizure. Both implanted mice tested at the same post-surgical intervals and unimplanted mice served as controls. Kindling significantly lowered HFE thresholds to generalized seizures and 30 days post-kindling in comparison with both control groups. Seizure expression differed at these timepoints, however. Only in the control group was the number of seizures significantly lower at 2 days, whereas b10 extended tonic seizures at 30 days post-kindling. Electrode implantation did not significantly alter HFE thresholds at either time point, but significantly altered seizure expression such that 7/7 exhibited tonic seizures at 30 days, but 0/7 at 2 days. Data suggest that damage caused by implantation of chronically indwelling electrodes significantly lowers the threshold for tonic seizure expression.

GP N HFE(2) Sz Type HFE(30) Sz type
Kindled 9 301±14 Chonic 264±15 Tonic
Implanted 7 403±28 Chronic 404±42 Tonic
Control 14 442±25 Chronic 422±93 Chronic
HFE(day)= fluorode test-latency to generalized seizure (sec + SE)
Sz type= seizure type: clonic-forelimb clonus; tonic-forelimb/clonus tonic

596.12 DIFFERENTIAL CHANGES IN THE ACTIVITIES OF MULTIPLE PROTEIN KINASE C SUBSPECIES IN THE HIPPOCAMPAL-KINDLED RAT. Kazutoshi Akiyama*, Mitsuhiro Ono and Kimiyo Tsuzuki and Shigehito Kukudo.*

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In previous studies we demonstrated that the membrane-associated protein kinase C (PKC) activities in the right and left hippocampus (HIP) of rats kindled (from the left HIP) significantly increased 4 weeks and 4 months after the last seizure compared with those in matched control rats (Daigen et al, Brain Res 545:131-136, 1991; Kohara et al, Brain Res 595:195-201, 1992). In the study, we investigated the long-lasting effect of HIPP-kindling on the membrane-associated activities of PKC subspecies in the bilateral HIP in 4 weeks and 4 weeks after the last kindling and the kindling had been induced by the kindling-associated activities of PKC subspecies were found to be subject to differential regulation. The activity of the α-subspecies was unchanged, whereas the reciprocal activities of the γ-subspecies were changed, respectively in the left- and right-subspecies. In the left-subspecies increased significantly, compared with the controls, one week (21%, P<0.0001), for the p-subspecies, and 23%, P<0.001 for the γ-subspecies) and 4 weeks (18%, P=0.001 for the p-subspecies, and 19%, P=0.001 for the γ-subspecies) after the last seizure. There were no significant differences in cytosolic PKC activity between the control and kindled groups for any subspecies, except for the γ-subspecies in the right-subspecies 4 weeks after the last seizure. These results suggest that activation of the PKC p- and γ-subspecies may play an important role in the enduring seizure susceptibility associated with kindling.

596.13 ACETAZOLAMIDE, A CARBONIC ANHYDRASE INHIBITOR, ACTS AS A PROCONVULSANT IN KINDLED RATS. L.J. Burdeett* and M.J. Lotharius.

Dept of Neurology, Graduate Hospital, Philadelphia, PA 19146.

Seizure activity alters extracellular pH which has been shown to influence NMDA and GABA-A-mediated currents. Acetazolamide inhibits the reversible hydration of CO2 by carbonic anhydrase, resulting in more pronounced synaptic transmission. The possibility that pH modulation of synaptic transmission may be affected by repetitive seizure activity (kindling model) was tested by comparing input/output (I/O) and paired pulse depression (20 and 200 ms interpulse intervals) functions recorded from the dentate gyrus of kindled rats before and after acetazolamide (10-20 mg/kg, p.o). Rats were kindled daily by perforant path stimulation (200-800 μAmps, 0.1 ms, 5 Hz, 9 sec) until 5 consecutive generalized seizures were obtained. Acetazolamide had no influence on the kindling-induced potentiation of I/O functions or of GABA-A-mediated early paired pulse depression. A dose-dependent decrease was observed, however, in late paired pulse depression. Rats were kindled for 5 Hz train duration required to elicit an afterdischarge (AD) also was significantly reduced by acetazolamide. We previously have shown that a failure of late inhibition precedes AD initiation, suggesting that the proconvulsant action of acetazolamide in kindled rats may result from the depression of late inhibition. Indirect support of a proconvulsant mechanism for NMDA also has been provided in earlier studies. Together with evidence that NMDA currents are increased at alkaline pH, these findings suggest that kindling may enhance the sensitivity of NMDA-mediated currents to alkaline shifts in the extracellular space, leading to a decrease in late inhibition and enhanced seizure susceptibility. Supported by NIH MH45961 to LJB.
597.1

DOWN-REGULATION OF AMYLOID PROTEIN PRECURSOR BY ANTISENSE OLIGONUCLEOTIDES REDUCES NEURONAL ADHESION TO LAMININ, E. Coccini1,2, L.C. Bartlett, P.F. Bartlett, D. Bailey, K. Beversluis1, and C.L. Masters.1 1Dept. Pathology, University of Melbourne, Vic., 3052, Australia. 2 Walter & Eliza Hall Institute for Medical Research, Parkville, Vic., 3052, Australia. 3 Center for Molecular Biology, University of Heidelberg, D-69120, Germany.

The Amyloid Protein Precursor (APP) is known as the source of βA4 amyloid, which forms plaques in brains of Alzheimer’s disease patients. APP forms a complex stabilized by alternatively spliced membrane bound and secreted glycoproteins and is a member of a large APP superfamily. APP isoforms are widely expressed, and in the brain products lacking exon 7 (the KPI domain) and containing exon 15 are most prevalent. The normal function of APP and its role in neurite extension and synaptogenesis is not yet clear. We are investigating the function of APP in neurons using phospholipase A2 antisense oligonucleotides. We find by cell adhesion assay that, after 24-48 hours, P2 mouse dissociated DRG neuronal cultures exposed to antisense APP oligonucleotides are less adherent to laminin (P<0.001) than cultures which are exposed to non-Antisense oligonucleotides of matched base composition. This change in adherence is not observed on other substrates (poly-L-lysine, fibrinogen, and matrigel). An effect on neuronal survival, neurite outgrowth, or neurite branching is not observed but this may be due to a lag time of APP down-regulation by the antisense oligonucleotides. Although neuritic networks are formed 16 hours after plating, the maximal effect on reduction in adhesion is after 48 hours, coincident with the patchy expression of APP on the surface of neurons. These data are consistent with a role for APP as a mediator of medium-term adhesion of neurons to extracellular matrix, and provide support for the specificity of an interaction of APP with laminin.

597.3

AMYLOID β PROTEIN PePTIDE (25-35) STIMULATES THE ACCUMULATION OF ALZHEIMER AMYLOID PROTEIN PRECURSOR VIA TAU PROTEIN KinASE I IN CULTURED HIPPOCAMPUS NEURON. A. Takashima1, H. Yamaguchi2, K. Ishigaku1, K. Nishimori1, T. Hoshino1, and K. Ohiri1.1 Research Institute of Life Sciences, 11 Minaminomiya, Machida-shi, Tokyo 194, 2 College of Medical Care and Technology, Gunma University, 3-39-15 Showa, Gunma, Japan.

Pathological changes of Alzheimer’s disease (AD) are characterized by cerebral cortical atrophy as a result of degeneration and loss of neurons. Typical histological lesions include numerous senile plaques composed of deposits of β-amyloid (Aβ) and neurofibrillary tangles consisting predominantly of ubiquitin and highly phosphorylated tau proteins. The exogenous application of synthesized amyloid β protein (Aβ) induced the neurotoxicity in the cultured hippocampal neurons. We recently found that some proteins induced by Aβ treatment leads to a programmed cell death in cultured hippocampal and that tau protein kinase I (TPKI) played a role in the Aβ induced neuronal death. Now we tried to identify the protein which was altered by Aβ treatment in the cultured hippocampal neurons. The level of amyloid protein precursor (APP) in cytoplasm increased by 10 folds of control level in response to exogenous application of Aβ peptide (25-35). The pretreatment of TPKI antisense oligonucleotide inhibited the Aβ induced APP accumulation and neuronal death. These results were interpreted as that TPKI regulated the increased APP accumulation in cytoplasm due to Aβ treatment, and that the increased APP accumulation in cultured hippocampus neuron might contribute to a process of Aβ induced neuronal death.

597.4

ECTO-PROTEIN KINASE IN BRAIN NEUROPS: A TARGET FOR ALZHEIMER’S β-AMYLOID PePTIDES. J. V. Hogan1, A. Patel2, J. A. Shimokawa1, H. A. Yang1, and J. A. Redin1.1 Shering-Plough Research, 2 Celltech, Bristol-Myers Squibb, Princeton, NJ 08543, USA.

Ecto-protein kinase (ectoPK) can serve as a direct target for neuroprotective agents that operate extracellularly. To study the effect of amyloid peptides on this activity, we have used primary neuronal cultures prepared from the telencephalon of 7-day chick embryos. The endogenous substrates of ectoPK on these neurons were identified as proteins with MW of 116K, 105K, 67K, 17K, 13K and 12K. The surface phosphorylation of the 12K and 13K proteins is catalyzed by an ectoPK with the specificity of PKC, and is maximal during de-novo neurite outgrowth. The β-amyloid peptide 1-40, when used at neurotoxic concentrations, inhibited the phosphorylation of the 12K and 13K surface proteins (over 80% inhibition of the phosphorylation of the 13K protein by 0.5µM β-amyloid peptide 1-40). At the same concentration, the β-amyloid peptide 1-26, which does not have neurotoxic effects, did not inhibit this phosphorylation. On the other hand, the neurotrophic β-amyloid peptide 25-35 selectively inhibited the phosphorylation of the 12K and 13K proteins by ecto-PK at the same concentration range (0.1-25µM) in which it produces neurodegeneration in cultured neurons. At nanomolar, non-neurotoxic concentrations, β-amyloid 25-35 stimulated this activity. Thus, the direct regulation of extracellular- PKC can mediate both the neurotrophic and neurodegenerative actions of β-amyloid peptides. Supported by HD20788
597.5

FURTHER ANALYSIS OF THE BRAIN Na+/Ca2+ EXCHANGER IN ALZHEIMER’S DISEASE. R.A. Colvin*, N. Davis, A. Wu, C.A. Murpho, and J. Leranth. Department of Biological Sciences, Ohio University College of Osteopathic Medicine, Athens, OH 45701 USA.

The Na+/Ca2+ exchanger was characterized in plasma membrane vesicles derived from frontal and temporal cortex and cerebellum of control and Alzheimer’s disease postmortem tissue. Exchanger activity was defined as the change in vesicular Ca2+ content seen after Na+ loaded vesicles were diluted into choline buffer. The time course of changes in Ca2+ content after dilution was similar in three regions of control brain, both frontal and temporal cortex vesicles showed elevated Ca2+ content, most evident as an increased peak Ca2+ content at 2 minutes. The AD cerebellar cortex time course was similar to control but did not show as elevated peak content. No differences were seen in the passive permeability to Ca2+ when comparing plasma membrane vesicles prepared from control and AD brain. Vesicles from the frontal and temporal cortex of AD brain showed increase in the initial velocity of Ca2+ uptake when compared to control brain, whereas, the cerebellum did not. There were no significant effects of AD on the Ki for Ca2+ activation of the initial velocity. Ca2+ influx measured during the rise in vesicular Ca2+ content was elevated in vesicles from AD temporal cortex when compared to control. Two known inhibitors (exchange inhibition peptide and dichloroethene) of the cerebral Na+/Ca2+ exchanger inhibited the human brain exchanger equally well in control and AD vesicles. An antibody to the cardiac exchanger was used to determine the molecular weight of the human brain Na+/Ca2+ exchanger. The molecular weight determinations from western blots showed identical molecular weights of 100-110 kDa in both AD and control vesicle preparations. The data suggest that increased Na+/Ca2+ exchange activity in AD brain is related to the causative factors of neurodegeneration and lends support to the “Ca2+ hypothesis of AD.”

597.6

THE INHIBITORY EFFECT OF B-AMYLLOID PEPTIDE ON RAT BRAIN NA+/CA2+ EXCHANGE. Anous Wu, Robert A. Colvin and Vasudeopal Jayasunder. Dept. Biological Sciences, Ohio University College of Osteopathic Medicine, Athens, OH 45701 USA.

These studies were performed to determine the effect of addition of Alzheimer’s disease β-amyloid peptide (B-AP) on Na+/Ca2+ exchange activity. Na+/Ca2+ exchange activity was measured from membrane vesicles (MV) isolated from rat brain. The effect of synthetic peptide B-AP,38-42 and scrambled (B-AP38-42) was examined. PMV were preincubated with each peptide for 15 minutes (4°C) before assay. Two mM of AD, 10 mM of B-AP38-42 and 15 mM of Ca2+ were included. Na+/Ca2+ uptake was initiated by diluting PMV 20 fold with buffer containing either 157 mM NaCl and 157 mM cholineCl and Ca2+. Ca2+ uptake was terminated by addition of 5 mM LaCl3 and rapid filtration. B-AP38-42 inhibited Na+/Ca2+ uptake with an IC50 of 800 μM (concentration measured in the preincubation buffer) but had no effect on the Na+−dependent Ca2+ influx. When B-AP38-42 was added in the dilution buffer instead of during the preincubation step, the same inhibitory effect was observed. No inhibitory effect was seen when B-AP38-42 was tested under both conditions. The inhibitory effect of B-AP38-42 could not be overcome by increasing Ca2+ concentration. Analysis of unidirectional flux for Ca2+ during the initial phase of rapid uptake or after a stable plateau had been obtained showed that 800 μM B-AP38-42 inhibited both Na+− and inhibts Na+/Ca2+ influx and efflux with the same efficacy (approximately 50 % inhibition) during both time periods. These results suggest that the inhibitory effect of B-AP38-42 is irreversible, and that it inhibits both Na+/Ca2+ exchange and Ca2+/Ca2+ exchange activity by directly interacting with the exchanger.

597.7


One of the hallmarks of Alzheimer’s disease (AD) is the presence of extracellular senile plaques composed primarily of aggregated amyloid β-peptide (AβP) surrounded typically by dystrophic neurites. It has been hypothesized that the aggregate form of AβP may induce toxicity on neurons by forming membrane channels thereby destabilizing neuronal Ca2+ homeostasis. We have used patch-clamp recording techniques to study the effects of AβP (1-40) on calcium currents in differentiated mouse NIE-115 neuronal cells (Davidson et al., 1984). In whole-cell recordings, incubation of cells with AβP for 24 h significantly increased the maximal peak inward current from -201.8 pA to -352.0 pA, and shifted the voltage at peak current (V1/2) and the current activation value towards more positive potentials. For untreated cells, median V1/2 was 1.7 mV and Vmax was -28.9 mV vs. 10.5 mV and -24.7 mV in AβP-treated cells. Inactivation with the reverse sequence AβP(40-11) did not produce significant changes in the amplitude or kinetic behavior of inward current. At the single channel level, AβP added to the pipette increased the open probability of cation-conducting ion channels. As determined by cell viability counts, AβP1-40 had neurotoxic effects; within 24 h, addition of AβP reduced the number of cells by more than 50%. It is suggested that the neurotoxic effects of AβP may be mediated by its ability to form cation channels de novo and/or alter the activity of cation channels already present in the cell membrane. We are in the process of developing a liposomal model system with a lipid composition similar to that of the neoblastoma cell to analyze AβP’s membrane perturbation properties.

597.8

ELECTROPHYSIOLOGICAL STUDY OF AMYLOID β PROTEIN ACTIVITY IN RAT CORTICAL NEURONS. K. Pakuwanta*, M.P. Mattson, and N. Alzgör. Sanders-Brown Research Center on Aging and Department of Anatomy and Neurobiology, University of Kentucky, Lexington KY 40536.

The effect of amyloid β-protein 25-35 (AβP) was examined in neurons freshly dissociated from rat cortex using the nystatin-perforated patch-clamp technique. AβP at concentrations higher than 10−8 M induced a slow inward current, which manifested its toxicity, with an increase in membrane conductance at a holding potential of -30 mV. The time lag until the appearance of the effect of AβP shortened concentration-dependently, whereas the increase in membrane conductance did not. When the extracellular Na+ and Ca2+ and intracellular K+ were replaced by equimolar NMG, isothionate, and Cs+ respectively, the membrane conductance and the reversal potential were not affected. Even when Ca2+ was removed from the extra- and intra-cellular space by using an Ca2+−free external solution and an internal solution including BAPTA (20 mM), the effect of AβP did not alter. It is suggested that AβP elicits an inward current either by forming membrane pores as suggested by Antepi et al., or by causing dysfunction of ion channels as suggested by our recent finding that AβP can damage ion-motive ATPases by a free radical-mediated mechanism (see Mattson and Matison, this meeting).

597.9


Amyloid β protein (Aβ) is a major component of the plaques that are a hallmark of Alzheimer’s Disease. A large literature has shown that Aβ is neurotoxic, but little has been published on the mechanism through which Aβ kills neurons. Here we report that treatment of embryonic rat cortical cells in culture with Aβ (1-40) or Aβ (25-35) inactivates the ouabain sensitive ATPase. Cultures were exposed to Aβ (10μM-200μM), membranes were prepared, and ATPase activity was assayed in 96-well microtiter dishes using a method adapted from Rohn et al. (Biochem. 46:525-34). We found that there is a reduction in cell activity with no reduction in the non-ouabain sensitive, non-calcium dependent ATPase activity. Co-treatment of cultures with Vitamin E (50μM) blocks the Aβ effect in ATPase activity, indicating that mechanism of impairment is mediated by free radicals. By a separate assay we also find no reduction in the activity of the plasma membrane Na/Ca exchanger at concentrations of Aβ that are neurotoxic. We are currently examining the contribution of impairment of Na-K-ATPase activity by Aβ to loss of calcium homeostasis and cell death.

597.10

PROPERTIES OF CHLORIDE CHANNELS IN PC12 CELLS INDIRECTLY MODULATED BY ALZHEIMER’S DISEASE B-AMYLOID. R. Raport, R. Fukuyama, G. Ehrenstein, S.I. Rapoport and Z. Galzicki; LNS, NIA and CNB, NINDS, NIH, Bethesda, MD 20892.

A pathological feature of Alzheimer’s disease (AD) is the formation of plaques in the parenchyma and vasculature of the brain. β-Amyloid peptide (B-AP) is one of the defining components of these plaques. We have previously shown from cell-attached patch recordings that B-AP (1-40) increases the activity of channels inherent to PC12 cells (Peacock et al, 1994, Biophys J. 66:A40). We have now managed to characterize these channels further. Under conditions of symmetrical choline chloride (120 mM) and high calcium (10 mM CaCl2), in excised patches, we could resolve two main conductance levels of 100 and 300 pS. Both had reversal potentials (RP) around 0 mV under these conditions. Exchanging the major cation on the extracellular face to Na+ had no significant effect on the RP. However, reducing the chloride to 30 mM NaCl from 150 mM, by replacement with acetate resulted in a positive shift in the RP, irrespective of whether chlorine or Na+ was the major cation. This implicated chlorine as the charge carrier for this channel, but the shift was less than expected indicating positive conductance. The fact that both conductances were seen to be effected in the same way by these ion substitutions and that both levels were invariably seen in any one patch, means it is likely that we are observing one channel which can have differing conductance levels. The mechanism by which B-AP or patch excision increases the activity of this channel remains unclear. A possible mechanism may be linked to the ability of B-AP to interfere with calcium homeostasis. Alternatively, disruption of cytoskeletal components linked to the channel may be involved.
NO APPARENT EFFECT OF \(\beta\)-AMYLLOID PEPTIDES ON M1 MUSCARINIC RECEPTOR BINDING OR FUNCTION. C.M. Rever and D.D. Flynn*, Dept. of Pharmacology, Univ. of Miami School of Medicine, Miami, FL 33101.

While acerelorated \(\beta\)-amyloid (\(\beta\)A) deposition has been suggested to play an early and critical role in the pathogenesis of Alzheimer's disease (AD), the precise cellular effects of \(\beta\)A are unclear. The complexity of \(\beta\)A actions is evidenced by the opposing neurotrophic and neurotoxic actions which are dependent on peptide concentration and the presence of soluble vs. fibrillar forms. On the basis of limited sequence homology to substance P and tachykinin peptides, \(\beta\)A has been suggested to interact with tachykinin receptors and function as an allosteric modulator of the ionotropic NMDA receptor. Substrate P-related peptides also appear to function as G protein antagonists, uncoupling receptor-G protein-mediated responses. Conical Mi muscarinic receptor (MR) high-affinity agonist binding and receptor-stimulated G protein-mediated phospholipase C activity are diminished in AD, resulting from an apparent receptor-G protein uncoupling. In order to test the hypothesis that \(\beta\)A may function as a MR-G protein "uncoupler" in AD, we have assessed the effects of amyloid peptides on MR binding and functional activity. \(\beta\)A peptides (1-40, 25-35, 0.01-100 \(\mu\)M) had no effect on binding of the muscarinic antagonist, \(\text{H}^3\)-NMS, to membranes from CHO cells transfected with the MR receptor, when incubated in vitro for up to 18 hr at 4\(^\circ\) C or for 2 hr at 25\(^\circ\) C, or in culture for 20 hr. Agonist binding as well as basal and carbachol-stimulated \(\text{H}^3\)-GTP\(\gamma\)S binding were also unaffected by peptide under all conditions tested. These findings suggest that \(\beta\)A does not antagonize MR-G protein interactions and that MR dysfunction in AD is not a consequence of excessive \(\beta\)A accumulation. (Funded by NS19905 & The Alzheimer's Association)
602.1

We previously reported that well-trained Parkinsonian subjects can show normal accuracy in a simple pointing task even if they are deprived of information about their unfolding trajectories and knowledge of the results of their movements (Neurosci Abstr 18:935). It has been often observed that Parkinsonian subjects require such information to sustain normal performance. However, such dependence might be due to task complexity or to the need to learn an unfamiliar transform. We therefore divided four groups of subjects: practiced normals (PN, N=3), practiced Parkinsonians (FP, N=1), unpracticed normals (UN, N=3), and unpracticed Parkinsonian subjects (UP, N=4). After a brief practice at making movements to match four computer screen targets requiring 8 to 32 cm excursions of the shaped arm over a digitizing tablet, subjects were required to respond to randomized targets in two experimental blocks. In the first, they saw the target throughout each trial, were given instantaneous feedback, and received knowledge of results. In the second, none of this information was provided. Both NP and FP subjects sustained accuracy when deprived of information, but neither UN nor UP subjects did so: both groups undershot the larger target distances, the UP subjects much more so (for the largest target, UP, 47.5% vs. UN, 26.3% and PN, 10.7%). We conclude that Parkinsonian patients have a relative inability to master a sensorimotor transform and perform under reduced information conditions rather than an absolute inability to do so. This research supported by the Dep. of Veterans Affairs Medical Research Council.

602.3
DECREASED [123I]-I-B-CIT SPECT STRIATAL UP TAKES CORRELATES WITH DISEASE SEVERITY IN IDIOPATHIC PARKINSON'S DISEASE. J. Seibyl, K. Marinkovic, E. T. Hauser, L. J. Zoghbi, R. Baloh, and R. Hoffer. Intra. Departments of Diagnostic Radiology, Neurology, and Psychiatry, Yale University School of Medicine, New Haven, CT, 06510-USA.

Our previous studies have utilized SPECT to demonstrate decreased [123I]-I-B-CIT striatal uptake in idiopathic Parkinson disease patients (PD). The present study extends this preliminary work by examining: 1) the test-retest reproducibility of two SPECT measures (ratio specific striatal uptake/non-specific uptake (V[S]/V[NS]) and striatal uptake expressed as the percentage of injected dose) in healthy subjects and, 2) striatal binding using these measures in PD patients of varying disease severity.

Methods: For the test-retest reproducibility study 7 healthy subjects (age range 19-74) had SPECT brain scans at 18, 21, and 24 hours following the first scan, at a random injection of 10 mCi [123I]-I-B-CIT. Repeat SPECT scans were performed 7-14 days following the first examination. For the PD severity study, a total of 26 idiopathic PD patients (age range 41-78, Hoehn-Yahr stages 1-5) had SPECT scans at 18, 21, and 24 hours after i.v. injection of 10 mCi of [123I]-I-B-CIT. The two measures were age-corrected based on our healthy control population and correlated with Hoehn-Yahr and total Unified Parkinson's Disease Rating Scale (UPDRS) scores of the patients.

Results: The healthy subject study demonstrated excellent test-retest reproducibility of both outcome measures with a mean 6.8 and 6.7% differences in V[S]/V[NS] and percent striatal uptake, respectively. In the PD study binding abnormality was correlated with both Hoehn-Yahr ratings and total UPDRS score for striatal V[S]/V[NS] (r=0.354 p=0.0016 and r=0.408 p=0.0004, respectively) and percent striatal uptake (r=0.33 p=0.0081 and r=0.469 p=0.001, respectively). These data suggest both [123I]-I-B-CIT SPECT outcome measures have a high degree of within-subject reproducibility and are strongly correlated with disease severity in idiopathic PD.

602.5

[123I]-I-B-CIT (2-carboxyhexyl-3-4-(iodo-phenyl)propy) binds dopamine (DA) transporter sites with high affinity and may assess DA-related behavioral function better than the tritium-labeled I-B-CIT SPECT studies, 18 hours after [123I]-I-B-CIT administration in African green monkeys, correlated with anatomical regional averages of positronem DA concentrations ([123I]-I-B-CIT SPECT) and with a detailed study of parkinsonian patients ([8-13]-I-B-CIT SPECT).

We obtained SPECT scans 18 hours after [123I]-I-B-CIT SPECT administration in African green monkeys before and after they received intrasural grafts of fetal mesencephalic tissue. Percent of administered [123I]-I-B-CIT dose was determined in a region of interest including the striatum and a circumferential background region.

Developing grafts were identified by 1% [123I]-I-B-CIT SPECT in vivo and confirmed by autoradiography and tissue histological immunochemistry. Over a 7 month period, mean changes in per cent administered [123I]-I-B-CIT dose in 4 untreated normals and in 4 normal monkeys (4 graft treated) [123I]-I-B-CIT depleted monkeys were significant. Four graft-treated monkeys with histologically confirmed grafts showed mean increases of 70% ± 22 SD. The largest increases corresponded with histological evidence of the most robust grafts. I-B-CIT-depleted monkeys were non-significant. Four graft treated monkeys with histologically confirmed grafts showed mean increases of 70% ± 22 SD. The largest increases corresponded with histological evidence of the most robust grafts. I-B-CIT-depleted monkeys were non-significant. Four graft treated monkeys with histologically confirmed grafts showed mean increases of 70% ± 22 SD. The largest increases corresponded with histological evidence of the most robust grafts. I-B-CIT-depleted monkeys were non-significant. Four graft treated monkeys with histologically confirmed grafts showed mean increases of 70% ± 22 SD. The largest increases corresponded with histological evidence of the most robust grafts. I-B-CIT-depleted monkeys were non-significant. Four graft treated monkeys with histologically confirmed grafts showed mean increases of 70% ± 22 SD. The largest increases corresponded with histological evidence of the most robust grafts. I-B-CIT-depleted monkeys were non-significant. Four graft treated monkeys with histologically confirmed grafts showed mean increases of 70% ± 22 SD. The largest increases corresponded with histological evidence of the most robust grafts. I-B-CIT-depleted monkeys were non-significant. Four graft treated monkeys with histologically confirmed grafts showed mean increases of 70% ± 22 SD. The largest increases corresponded with histological evidence of the most robust grafts.
602.7

The discovery that a methyl-1,4-phenol 1,2,3,6-tetrahydropyridine suggests that endogenous and xenogenous neurotoxins may cause cell death of dopaminergic neurons in the substantia nigra and thus induce in the Parkinson's disease in humans. As neurotoxin candidates, dopamine-derived 6,7-dihydroxy-1,2,3,6-tetrahydroisoquinolines (DHTIQs) have been examined on their effects on dopaminergic neurons. Out of DHTIQs, 1-methyl-DHTIQ (M-DHTIQ) is methylated to 1,2,3-(3,4-dihydropyridine, primer of which is taken to be involved in 1,2,3,6-dihydroxyisoquinolinium ion (1,2-DimDMHetO*). 1-MeDHTIQ and its derivatives were injected into the striatum of male Sprague-Dawley rat, and the biochemical, behavioral, and pathological changes were examined. Both (R) and (S)enantiomer of 1-MeDHTIQ and 1/S(2)-DimDMO* did not induce any behavioral change by a single administration. 1R(2)-DMO* induced electrophysiological changes to the rat: akinesis, bradykinesia, postural abnormality and rigidity were detected. The behavioral changes were transient; they disappeared several hours after the injection. Three day after the injection, the rat was sacrificed and DHTIQ derivatives and monoamines were analyzed by HPLC-electrochemical and fluorometric detection. Biochemical analysis showed that 1R(2)-DiMeDHTIQ was detected in the striatum, and larger amount of the oxidized product, 1,2-DimMO* was identified in the substantia nigra in addition to the striatum. Dopamine, noradrenaline, as their metabolites were found to be reduced in the striatum and the substantia nigra. The injection of 1R(2)-DiMeDHTIQ induced massive necrosis in the rat brain. Using a human dopaminergic neuraloblastoma SH-SYSY cells, only 1R(2)-DMO* was confirmed to be taken up to the cells by dopamine transporter, which is a high-affinity DHTIQ derivative. These results suggest that 1R(2)-DmDMO* may be neurotoxic to dopaminergic neurons.

602.8
NICOTINE PREVENTS EXPERIMENTAL PARKINSONISM IN RODENTS F. Vaglini, F. Facetti, F. Fontana1, R. Maggio, G.U. Corini Instituto of Pharmacology, School of Medicine, University of Pavia, ITALY

We have demonstrated that diethylthiodicarboxamate (DCC) enhances MPTP toxicity in mice. This combined treatment induces a more marked depletion of striatal dopamine (DA) and a severe loss of tyrosine hydroxylase (TH) positive perikarya in Substantia Nigra pars compacta (SNpc) (Corsitwi et al., Eur. J. Pharmacol.,119,127; 1985). This represents a reliable model of experimental parkinsonism in rodents on which neuroprotective molecules can be evaluated. For instance, it is now well established that non-competitive NMDA antagonists (MK-801, dextromethorphan) provide a complete protection on DDC enhancement of MPTP toxicity.

In the present study we used this model in order to evaluate the effect of nicotine, on experimental parkinsonism. (-) Nicotine (1mg/Kg, s.c.) administered three times (90 and 30 min before and 30 min after MPTP), completely prevented both the marked depletion of striatal dopamine and the severe loss of TH positive perikarya in SNpc induced by DCC+MPTP. Nicotine provided this protective effect without reducing striatal MPP+ levels measured at several time intervals after MPTP administration.

Despite previous contrasting findings, our data suggest that nicotine could be responsible of the reduced prevalence of Parkinson's disease among smokers.

602.9
ELEVATION OF NEURONAL MAO-B ACTIVITY IN A TRANSGENIC MOUSE MODEL DOES NOT INCREASE SENSITIVITY TO THE NEUROTOXIN MPTP.

J.K. Anderson1, D.M. Trim, O. Issacson, M.F. Beal, and X.O. Breikfield, Neurology Service and Neurosurgery Service, Massachusetts Hospital, Charlestown, MA, Harvard Medical School, Boston, MA, and Neuroregeneration Laboratory, McLean Hospital, Belmont, MA

To examine whether expressing high levels of monoamine oxidase (MAO-B) activity abnormally in neurons results in increased sensitivity of dopaminergic neurons to the neurotoxin 1-methyl-4-phenol-1,2,3,6-tetrahydropyridine (MPTP), 8 week old transgenic mice expressing high neuronal levels of MAO-B were compared with age-matched nontransgenic littermates following i.p. injections of 30 mg/kg body weight of the protoxin. Levels of striatal dopamine (DA) and its metabolite 3,4 dihydroxyphenylacetic acid (DOPAC), as well as tyrosine hydroxylase (TH)- immunopositive cell number in the substantia nigra (SN) were compared 1 week later between transgenics and controls. No difference was found in any of these parameters, indicating that high neuronal MAO-B levels does not cause increased sensitivity to MPTP, and therefore neither conversion of MPTP to its active form, 1-methyl-4-phenyl pyridinium (MPP+) by MAO-B nor MPP+ uptake by the dopaminergic transporter are likely to be the rate-limiting step in the toxicity of this compound.

602.10
AAV-MEDIATED TRANSFER OF TYROSINE HYDROXYLASE AND AROMATIC AMINO ACID DECARBOXYLASE GENES INTO THE PRIMATE BRAIN: A DIRECT GENE THERAPY APPROACH TO PARKINSON'S DISEASE

M.J. Durairaj1, R.G. Kaplan Jr., P. Leone, C. Xiao, J.D. Elworth, H.R. Roth2, R. Shakin1, D.P. Redmond1, R.K. O'Malley, R. Vaglini, 1 Yale Univ Sch Med, New Haven, CT 06520; 2Rockefeller Univ, New York, NY 10021; 3Vahab Univ Sch Med, St Louis, MO 63110; 4UNC, Chapel Hill, NC 27599; 5Univ Hlth Sci/Chicago Med Sch, Chicago, IL 60604

AAV is a defective human parvovirus which has several major advantages for gene transfer in the CNS including non-pathogenicity, targeted integration and stable expression in post-mitotic cells including neurons (Kapliit et al., Soc. Neurosci. Abstr., 1994). Moreover, AAV vectors are devoid of viral genes and a novel packaging method to generate helper-free stocks is established. This vector system may have application therefore for gene therapy of neurological disorders. The human tyrosine hydroxylase (form ID) cDNA was truncated to 1.1 kb by deletion of the N-terminal regulatory region and a FLAG epitope added (FLAG-THA). FLAG-THA together with the human aromatic amino acid decarboxylase gene was packaged into AAV using the rnc virus IRES sequence to form a bicistronic construct (AAVhTHA/IRESc). AAVhTHA/IRESc transduced Hek 293 cell cultures released dopamine into the medium at high concentrations (3.1±10-10 cell-1 min-1). AAVhTHA/IRESc was stereotactically injected into the partially denervated caudate of MPTP-treated African green monkeys. 10 days following injection, two animals were sacrificed and expression of the vector encoded TH was determined using a FLA antibody. FLAG-IR neurons were observed around the injection sites. These data suggest that AAV can transduce neurons in the primate brain in vivo. If dopamine release can be increased by this strategy AAVhTHA/IRESc may have utility as a direct gene therapy approach to Parkinson's disease.

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SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
603.1 DEVELOPMENTALLY SPECIFIC RNA EDITING IN AMPA RECEPTOR SUBUNITS GluR-B, -C, -D, H. Lomeli1, T. Melcher1, T. Higaki2, A. Bach2, G. Kehr3, M. Higuchi3, B. Spranger3, H. Monyer1 and P.H. Seeburg1Center for Molecular Biology (ZMBH), University of Heidelberg, and BASF Inc., Tübingen, Germany

In glutamate-operated receptor channels of the non-NMDA type in cerebral cortex, several functionally critical channel residues not encoded by the genes for the channel subunits are introduced via nuclear RNA editing. This process operates on the preexisting sense transcripts to substitute glycine residues for arginine residues in guanosines. Positional selectivity and efficiency of RNA-editing appear to rest on a double-stranded RNA structure formed by the to-be-edited complementary sequence and a complementary sequence in the proximal intron [Higuchi et al., Cell 52, 1361; 1993]. Detailed cdNA analysis has now revealed that AMPA receptor subunits GluR-B, -C and -D share a position either occupied by arginine (gene-encoded) or by glycine, the latter residue being introduced by site specific adenosine to guanosine RNA editing. As for the GlR-B site editing of GluR-B, editing at this newly-identified site is mediated by an intrinsic sequence element present in the GluR-B, -C, and -D genes but is absent in the Glur-A gene. A PCR-supported analysis in brains of staged rats showed that RNA editing at the new site progresses in extent from approximately 10% at E14 to >80% by P12. Furthermore, single-cell PCR analysis showed that subunits expressed in one cell can be edited to different degrees.

603.3 REGULATION OF N-METHYL-D-ASPARTATE RECEPTOR SUBUNITS EXPRESSION IN CEREBRAL NEURONS. B. Lambrolakis, B. Audsitan, S. Chartaj, J. Russet1 and F. Citcele1, IAF CNRS, 19198 Gif/Yvette, France; Lab. Neurobio, CNRS URA 1507, Paris, France and Ips, Physiol., 12,14 Genève 4, Switzerland.

The N-methyl-D-aspartate (NMDA) subtype of glutamate receptors is an oligomer formed of different subunits named NR1 and NR2A, NR2B, C and D the combination of which determines the functional properties. We have used rat cerebral slice cultures to study the involvement of spontaneous activity and extracellular inputs on changes of NR2A-C expression observed during the postnatal development of granule and Purkinje cells. The functional properties of the NMDA receptors formed by A-C mRNAs were correlated in single neurons by coupling patch-clamp recording and reverse transcription followed by PCR. Granule cells grown in standard culture conditions expressed mainly NR2A mRNAs when examined after 15 to 40 days in vitro. Consistent with this observation, their responses to NMDA were poorly induced by 5 mM tetrodotoxin, a non-competitive antagonist which discriminates NR2A and NR2B subunits in expression systems. The NR2C subunit, abundantly expressed in vivo by adult granule cells, was only rarely detected in slice cultures even when excitatory synapses were formed between granule cells and mossy fibers originating from co-cultured brainstem explants. In cerebellar cultures which had been chronically exposed to 300 microM glutamate during 15 to 35 days in vitro to reduce spontaneous activity, granule cells expressed predominantly NR2B subunits (as it is the case only during the first two postnatal weeks in vivo) and their responses to NMDA were largely inhibited by 3 mM tetrodotoxin. These results provide evidence that the expression of NR2A and B subunits is regulated through an activity-dependent mechanism leading to the formation of NMDA receptors with different pharmacological properties. Finally, in none of the experimental conditions described above, we could detect any NR2 subunit in Purkinje cells, although NR1 was constantly expressed. This was in agreement with the absence of NRD responses.

603.5 GENERATION OF ANTIBODIES SPECIFIC FOR PHOSPHORYLATED NMDA RECEPTORS. W.G. Tingley and R.L. Huganir*. Department of Neuroscience, H.H.M.I., Johns Hopkins University School of Medicine, Baltimore, MD 21205.

A variety of neuronal processes, including synaptic plasticity, synaptic gene expression, and excitotoxic neuronal damage, involve N-methyl-D-aspartate (NMDA) receptors. NMDA receptors in the brain are thought to be composed of multiple subunits, including at least one NR1 subunit and one or more NR2 subunits. A variety of studies have suggested that NMDA receptors are regulated by protein phosphorylation. We have shown recently that protein kinase C (PKC) directly phosphorylates NR1 near its C-terminus, in a region regulated by alternative splicing. For this study, we have generated multiple anti-phosphoepitope antibodies recognizing the NR1 protein only when it is phosphorylated. Immunocytochemical and immunoblot experiments with these antibodies indicate that CAMP-dependent protein kinase A (PKA), like PKC, phosphorylates NR1 near its C-terminus. However, PKA and PKC phosphorylate different serine residues within this region. In addition, phosphorylation in this region appears to regulate the subcellular distribution of NR1 protein expressed in HEK-293 cells. These antibodies should prove useful for monitoring the phosphorylation state of NMDA receptors in vivo, including the induction and maintenance of LTP in the hippocampus.

603.6 BIOCHEMICAL AND TOPOLOGICAL ANALYSIS OF THE NMDA RECEPTOR. Andreas K.E., Köhle, Silvia Correa, Sabine Sydow, Ines Bors and Joachim Spira *, Max Planck Institute for Experimental Medicine, Molecular Neuroendocrinology, Heinitz-Rein-Str. 3, 37075 Göttingen, Germany

We are interested in the structure/function relationship of the NMDA receptor that is thought to be important in learning, memory and several neurological disorders. We want to analyze the properties of distinct subunit compositions by biochemical and electrophysiological techniques in a defined artificial system.

Fourteen regio specific antibodies have been produced to characterize the NMDA receptor subunits NR1, NR2A and NR2C. Eighteen cell lines expressing single NMDA receptor subunits (NR1, NR1B, NR2A and NR2C) or subunit combinations (NR1A/B, NR2A/B, NR2C/D, NR2D/D) were generated.

The double transfected cell lines showed distinct morphologies and were sensitive to high concentrations of glycine and NMDA. In the presence of the NMDA receptor antagonists, eight cell lines showed a strong upregulation when the NR2 protein was present, whereas the NR2 protein amount was similar in the presence or absence of NMDA in this defined artificial system, in which standard proliferation is achieved by a combination of low serum and high glucose. These findings could be explained by an increased degradation of subunits or a translational regulation of NR2 protein expression.

The transmembrane topology of the NR2C subunit was investigated with antibodies specific for the N-term (NR2C250) and C-term (NR2C548) epitopes. NR2C250 was detected on the surface of the NR2C1 or NR2C2-transfected HEK293 cells. Anti NR2C-D stained the NR2C subunit only in punctated or stunted parasitized cells. These results are direct experimental evidence that the four transmembrane domain model suggested first for the cloning of the cholinergic muscarinic receptor models suggesting a cytosolic localization of the entire C-terminal domain are not in agreement with these results.

Another approach for the direct protein chemical characterization of the NR1b subunit is based on its overexpression in the baculovirus system. December the recombinant protein is probably present in inclusion bodies and found at a concentration of approximately 1 mg per liter of culture medium.
603.7
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The transmembrane domain (TMD) topology of glutamate receptors (GluRs) is incompletely understood. Although GluRs are commonly modeled after the nicotinic acetylcholine receptor (nAChR), no direct evidence is available for the presumed 2TMD plus 4TMD model with four transmembrane (TMD) and an intracellular-forming TMD II, and even the assignment of the four hydrophobic domains to TMDs is made in different ways (see Hollmann & Heinemann, Anita. Rev. Neurosci. 1994.17:31-108).

We set out to obtain experimental evidence for extracellular or intracellular localization of the various domains of the GluR1 protein expressed in Xenopus oocytes. We used site-directed mutagenesis to introduce N-glycosylation sites (N-X-S or N-X-T) in C-terminal positions throughout the receptor protein. N-glycosylation sites were created in the N-terminal and C-terminal domains as well as in those domains located in between hypothetical TMDs. Mutant GluR1 proteins expressed by Xenopus oocytes were analyzed in SDS gel shift assays, for an increase in apparent molecular weight caused by the attachment of carbohydrate side chains. GluR1 was visualized on Western blots of SDS gels using polyclonal anti-GluR1 antisera (kindly provided by Bob Wenthold and Richard Huganir). Upward shifts of mutant receptors vs. wild type receptor were interpreted as evidence for the extracellular or intracellular localization of the introduced N-glycosylation site.

The results of the gel shift assays demonstrate that the N-terminus of GluR1 is indeed extracellular as assumed in all models proposed to date, while the C-terminal is intracellular. Our data also indicate that the domain between TMD III and TMD IV, which generally was assumed to constitute a large intracellular loop, is actually extracellular. The topology in between TMD I and TMD III is currently under investigation.

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603.8
IDENTIFICATION OF A CAM-KINASE II REGULATORY PHOSPHORYLATION SITE IN NON-NDMA GLUTAMATE RECEIVERS.
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Glutamate receptor (GluR) ion channels are localized in postsynaptic densities with CamKII, an enzyme which has been previously shown to phosphorylate and stabilize the non-NDMA ion currents in cultured rat hippocampal neurons (McGlade-McCulloh et al., 1990, Nature 362:640). We wanted to extend these findings by demonstrating the regulatory effect of CamKII on expressed non-NDMA (i.e., AMPA-type) GluRs, and to identify the regulatory site phosphorylated by CamKII. In this study, the intracellular application of activated CamKII produced an increased sensitivity to kainate in both recombinant oocytes expressing GluR1 and in Xenopus oocytes expressing GluR1 enhanced the GluR current activated by kainate. This effect of CamKII on GluR1 was further investigated in the Xenopus oocyte/GluR1 system which gave a 1.9-fold increase in kainate current with no effect on the sensitivity of the receptor to kainate. When Ser672, which is located in the major intracellular loop of GluR1, was mutated to Ala, the expressed mutant no longer responded to the CamKII-induced enhancement of kainate current but was normal with respect to current-voltage relationship and kainate sensitivity. Since all non-NDMA GluR ion channels have a Ser in an analogous position, this regulatory phosphorylation site may be of general importance in enhancing post synaptic responsiveness as occurs in long-term potentiation.

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603.9
IBOTENIC ACID ACTIVATES A CA2+ MODULATED CHANNEL IN CERESELLAR P Purkinje NEURONS.

Ibotenic acid acts as an ionotropic (NDMA and nonNDMA) and metabotropic excitatory amino acid agonist in vertebrate brain. In mouse cultured Purkinje neurons, cells which do not express NDMA, ibotenic acid produced relatively small inward currents at negative membrane potentials compared to AMPA and kainate. In whole cell recordings, ibotenate-activated currents were observed to increase in magnitude when the extracellular Ca2+ was increased from 1 to 10 mM, suggesting that these channels are modulated by Ca2+ ions. To determine which of the non-NDMA channels might underlie this effect, the changes in Ca2+ levels were monitored on ibotenate-activated single channel currents. The currents were examined in outside-out patches from Purkinje neurons. Recording pipettes typically contained 140 mM NaC1, 4 mM ATP-Mg, 10 mM Hepes-Na (pH 7.2) and 2 mM EGTA/1 mM CaC12, but occasionally 2 mM BAPTA/1 mM CaC12 in the inside-out solution (100 mM NaC1, 2.8 mM KCl, 10 mM Hepes-Na (pH 7.2)), ibotenate opened channels with conductances of 20-30 pS, with a reversed potential near 50 mV. Of these, 30 pS channel observed in more than 90% of patches from Purkinje cells, was studied in different Ca2+ levels from 1 mM to between 5 and 110 mM while substituting for Na+. In 5 mM CaC12 (the open probability (Po) of ibotenate channels increased 1.5-2 fold. Changing from 1 to 110 mM CaC12 (150 mM NaC1 to 0 Na+), Po increased 6 fold at -60 mV with relatively little change in channel conductance, the result being between a 3.5 and 5.5-fold increase in the net flux in the N direction. In time-averaged single channel recordings the mean current at -60 mV approximately doubled by increasing Ca2+ from 1 to 10 mM, similar result was obtained in whole cell recordings. These observations were made by increasing Ca2+ and, however, the possibility remains that the increase in Po is due to an increase in Ca2+.

603.11
HETEROERIC OLFACTORY CYCLIC NUCLEOTIDE-GATED CHANNELS: A NEW SUBUNIT THAT CONCOLORS INCREASED SENSITIVITY TO CAMP, J.L. Bradley, N. Davidson, H. Lester & K. Zyu.
Division of Biology, California Institute of Technology, Pasadena, CA 91125.

One mechanism of olfactory signal transduction involves the opening of cyclic nucleotide-gated (cng) channels. Cng channels in rat olfactory neurons are activated by cAMP in the low pM range and are outwardly rectifying. The previously cloned cng channel (ICOCN1), however, shows much lower cAMP sensitivity and very weak rectification when expressed in 293 cells. We have cloned another cng channel from rat olfactory epithelium cDNA library, ICOCN2 encodes a protein of 575 amino acids with 51% identity to ICOCN1. ICOCN2 does not form homomultimers when expressed alone. When ICOCN2 is coexpressed with ICOCN1, however, the resulting conductance is characterized by an outward rectification stronger than that observed with ICOCN1. By a CAMP sensitivity close to that of the native olfactory cng channel. ICOCN2 is expressed with ICOCN1, however, the resulting conductance is characterized by an outward rectification stronger than that observed with ICOCN1. In divalent-free conditions, the ICOCN1 channel is not fully activated by cAMP, suggesting that the native olfactory cng channel is a heteroneric channel. In the presence of cAMP, ICOCN2 blocks the inward current at ICOCN1 channels more strongly than at ICOCN1/ICOCN2. In divalent-free conditions of ICOCN1 channels are uninterrupted for tens of ms, while those of ICOCN1/ICOCN2 channels are flaky, especially at negative membrane potentials. This flaky behavior resembles the native channel. In all hybridizing, demonstrates that the two subunits are coexpressed in olfactory receptor neurons. These results indicate that the native olfactory cng channel is a heteroneric channel, and that the overall properties of olfactory channels are structurally and functionally analogous to those in rod photoreceptors.

Support: NIH, HHMI

603.10
ODORANT INDUCED PHOSPHORYLATION OF INOSITOL 1,4,5-TRIPHOSPHATE RECEPTOR IN Olfactory RECEPTOR NEURONS, J.L. Fujimoto,
John Hopkins University School of Medicine, Department of Neuroscience, Baltimore, MD 21205.

Both the cyclic adenosine 3',5'-monophosphate and cyclic guanosine 3',5'-monophosphate pathways are involved in olfactory signal transduction, which occurs in the cilia of olfactory receptor neurons. In olfactory receptor neuron, inositol 1,4,5-triphosphate (IP3) receptor is located at the surface plasma membrane of olfactory cilia and play roles in regulating calcium flux and the surface membranes. To explore its involvement in odorant signal transduction, the phosphorylation properties of IP3 receptor were studied in cultured olfactory sensory neurons. Odorant remarkably enhanced IP3 receptor phosphorylation with the peak at 2 minutes after stimulation. Further exposure of odorants induces dephosphorylation to the IP3 receptor levels. This result indicates the modification of IP3 receptor function in the late phase of odorant signal transduction. Dose-response curve revealed the maximal odorant effect at the concentration of 100 mM. These results may provide a mechanism whereby desensitization occurs in odorant perception.

603.12
ELECTROPHYSIOLOGICAL PROPERTIES OF LIGAND-GATED ATP RECEPTOR IN RAT AUTONOMIC NEURONES AND VAS DEFERENS SMOOTH MUSCLE. A. Sarparsv, B. Khabs and C. Memit.
Galo Institute for Molecular Biology, Geneva, Switzerland, 1228.

P2X purinoceptors exist in autonomic neurons and many vascular and visceral smooth muscle where they can mediate fast synaptic transduction by activating a cationic receptor-channel complex. We used whole-cell recording techniques to compare properties of this receptor in rat nodose and SCG neurons as well as in isolated rat vas deferens smooth muscle. Fast-flow application (0.5-2 s duration) of ATP, 0.1mM ATP and 2mM ATP evoked rapid inward currents in all cells; marked desensitization of the inward currents was observed in smooth muscle but not in neurons. Calcium permeability was significant in vas deferens and nodose neurons (PCaPNa = 5) but minimal in SCG neurons. Inward rectification of the current was observed in all cells but was most pronounced in vas deferens and SCG. In all neurons, agonist was 2mM ATP > ATP > 0.1mM ATP. Half-maximal effective concentration (EC50) of ATP was about 1 µM for vas deferens and vas but 40 µM for SCG. 0.1mM ATP was a full agonist in smooth muscle but acted as a partial agonist in SCG. Properties of the ATP response in nodose and vas deferens were very similar those observed for the P2 receptor recently cloned from rat vas deferens (Valera et al., this meeting). The different pharmacological and physiological properties of the ATP response in SCG neurons suggests the presence of a distinct receptor subtype.
604.3


The aims of the present investigation were to provide evidence that in rat and guinea-pig cerebral cortical slices, \(\Delta^2\)Herotonin (\(\Delta^2\)H-T) release can be stimulated via glutamate receptors of the NMDA and non-NMDA type and that this release is modulated via presynaptic auto- and heteroreceptors. The slices were preincubated with \(\Delta^2\)H-T in the presence of mepiparte (in order to avoid false labeling of noradrenergic neurones) and superfused with Krebs' solution (Mg^2+ free in the experiments with NMDA). NMDA (0.1-1 mM) stimulated the overflow of \(\Delta^2\)H-T from slices of both species in a manner sensitive to blockade by 0.3 mM tetrodotoxin (TTX). 1.2 mM Mg^2+ (DL-\(\Delta^2\)-aminooxy-4-methyl-5-phosphono-3-pentanoic acid; a competitive NMDA receptor antagonist), 100 mM dicyclophone, 10-100 mM 5,7-dichlorokynurenic acid, 100-300 mM amphetamine and 0.1-0.1 mM (l)-propranolol (0.1-0.1 mM) also stimulated \(\Delta^2\)H-T overflow. The KA4-evoked overflow in the presence of 1.2 mM Mg^2+ was not affected by CGP 37849 and aracene, but was inhibited by 0.3 mM TTX and 100 mM CGP 37849 (6-cyano-7-nitroquinoxaline-2,3-dione; an antagonist of non-NMDA receptors). 5-Carboxamidotryptamine (1-100 mM; a 5-HT receptor agonist) and acetylcholine (1 and 10 mM) inhibited the NMDA-evoked overflow; these responses were antagonised by the 5-HT receptor antagonist methysergide and the 5-HT receptor antagonist idazoxan, respectively, which gave alone facilitated \(\Delta^2\)H-T overflow.

The experiments were performed using whole-cell techniques and the results presented are mean values ± S.E.M.

604.4

CALCULATING CHANNELS VI

604.1


Autophosphorylation of nonadrenaline release from cholinergic sympathetic neurons depends on \(\alpha\)-adrenergic inhibition of \(N\)-type calcium channels by pertussis toxin-sensitive \(G_{i/o}\) proteins. The present study investigates the signalling cascade between the \(\alpha\)-adrenergic stimulus and the release of noradrenaline.

A role of protein kinase C (PKC) in neurotransmitter modulation of calcium channels is generally indicated by in vitro experiments using analogues of phorbol esters or dicyclophosphate analogues. However, neither 4-phorbol-12,13-dibutyrate (PDB, 3\(\mu\)M) nor 4-phenacylbutyryl [Gly-Val-Glu(\(\alpha\)-Me)-Val-Lys(D emailing)-Arg(Drinking)-Phe-D-Pro-Val] [PDB (10\(\mu\)M) affected whole-cell calcium currents (ICa). Nevertheless, inclusion of a pseudophorbol peptide inhibitor of PKC (PCK 10\(\mu\)M, 100\(\mu\)M) in the pipette progressively reduced the inhibitory action of the \(\alpha\)-adrenergic agonist brevamine (10\(\mu\)M) upon ICa. When neurons were dialysed with PCK for 10 min, brevamine inhibition was 63% (24% to 96% control) in the absence of PCK and 58% (10% to 90% control) in the presence of 10\(\mu\)M PCK. These results revealed the involvement of a pseudophorbol peptide of PKC in the modulation of brevamine inhibition. In conclusion, the present results suggest an involvement of a pseudophorbol peptide of PKC in the modulation of brevamine inhibition. These results suggest that a pseudophorbol peptide of PKC may be involved in the modulation of brevamine inhibition.
CALCIUM CHANNEL MODULATION BY LECTINS. A. Golard*1, Howard Hughes Medical Institute, Dept. of Physiol. & Phrenology, Univ. of Washington, Seattle, WA 98195

Calcium currents were measured in dissociated chick sympathetic ganglia using the whole cell patch clamp technique. Lectins (1 μg) were applied to the local superfusion. All lectins tested reversibly inhibited Ca currents. Two types of inhibition were observed: a speeding of inactivation (SI) and a voltage-dependent inhibition (VDI) with slowing of the activation kinetics. When GTP-γ-S is substituted for GTP, VDI is abolished, while SI is still reversible. GDP-β-S largely suppresses VDI, but has little effect on SI. These results indicate that G-proteins are involved in VDI, not SI. Thus, VDI may be due to activation of G-protein-coupled receptors, while SI may be a direct effect on the channel. Lectins with high affinities for N-acetylglycosamine or α-D-glucose preferentially induce VDI, while lectins with α-D-galactose affinity produce SI. Differential effects of concanavalin-A and sucrose-con-A indicate that SI may be due to capping.

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604.11

MODE SHIFT OF N-TYPE CA\(^{2+}\) CHANNEL BY THE AUXILIARY SUBUNITS \(\alpha_2\) AND \(\beta\) Minou Wakanomat* and Yasuo Mor*. Dept. of Pharmacol. and Cell Biophys., Univ. of Cinti., Cinti, OH 45267-0575

\(\omega\)-Conotoxin-GVIA-sensitive N-type channels are inhibited by various neurotransmitters. Modulation is predominantly mediated by L-\(\omega\)-proteins which alter equilibrium among diverse functional behaviors. To gain a clear structural insight into functional diversity of N-type channels, we have investigated the influence of recombinitant N-type channels composed of various combinations of the pore-forming subunit \(\alpha\) (BIII) and the two additional subunits, \(\alpha_2\) and \(\beta\), in Xenopus expression systems in 40 mM Ba\(^{2+}\) showed that the BIII \(\alpha\) subunit alone elicited \(\omega\)-conotoxin-sensitive HVA Ba\(^{2+}\) current, with a time-to-peak (\(t_p\)) and decay time constant (\(\tau_d\)) of 16 and 215 ms, respectively, at +20 mV. Modulation of the BIII channel kinetics by \(\alpha_2\) subunits was strikingly different from that observed for the L-type channels, i.e., both \(t_p\) and \(\tau_d\) are increased in BIII (21 and 477 ms) but decreased in L-type. The \(\beta\) subunits shifted the current-voltage relationship in a hyperpolarizing direction by 8 mV as was observed for the L-type channels. Surprisingly, the effects of the \(\alpha_2\) subunit contrasted with and antagonized the effects of the \(\beta\) subunits (\(t_p=9, \tau_d=144\) ms for BIII+\(\alpha_2\); \(t_p=10, \tau_d=248\) ms for L-BIII+\(\beta\)). Single channel analysis revealed different patterns of opening for BIII, BIII+\(\alpha_2\), BIII+\(\beta\), and BIII+\(\alpha_2+\beta\), suggesting that different subunit combinations of calcium channel complexes give rise to different modes of gating dependent upon the association/disassociation of the subunits.

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605.1

DEVELOPMENT OF DIRECTIONAL SELECTIVITY IN TURTLE RETINA: PHYSIOLOGY AND MODEL. N. M. Grywacz* and E. Sherman, South-Kentwell Inst., 2232 Webster St., San Francisco, CA 94115

Directional selectivity requires a spatially asymmetric mechanism to mediate the larger responses to preferred- than to null-direction motions. What developmental processes mediate the breaking of symmetry in the retina? To study this development, we recorded extracellularly from turtle retinal ganglion cells during embryogenesis (Stages 22-26), the first 40 days post-hatching, and in adults. In particular, responses were made of the Staggers, a bursting burst of spikes in immature retinas and of the responses to edges of light moving in sixteen directions. The bursts occurred during Stage 22 until 2-4 weeks post-hatching. Light responses began at Stage 23, and early on, polar plots of the responses to motion were highly anisotropic, including orientation and directional selectivity. However, orientation selectivity reached a peak at incidence at hatching, while directional selectivity disappeared only to reappear in adults (after 40 days post-hatching). Receptive-field sizes and the incidence of isotropic cells stabilized 2-4 weeks post-hatching.

We built and trained computer simulations of a model system to account for the development of directional selectivity. In this model, the early anisotropy of the retina reflects immature, polarized dendritic layout, with directional selectivity being incidental to the dendrite's cable properties. As dendritic processes grow and branch, embryonic dendritic selectivity disappears, but orientation selectivity is maintained by a Hебbian process, which reinforces some of the early anisotropy. Later in development, the emergence of a specialized inhibitory synapse onto the dendritic inputs to some orientationally selective cells, transfers them onto weak directionally selective cells, which are then reinforced by a Hebbian process.

In conclusion, in increases in dimension of dendrites, their degree of polarization, and the spontaneous burst of spikes do not appear to account directly for the development of directional selectivity. Rather, it seems to require visual experience and/or the last stage of development of a specialized inhibitory synaptic drive.

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605.3

AXONAL BRANCH DYNAMICS: AN IN VITRO EXAMINATION OF THE APPLICATION OF HEBB'S HYPOTHESIS TO AXON MORPHOLOGY. S. Wint* and H.T. Cline, Dept. of Physiology, University of Iowa, Iowa City, IA and Cold Spring Harbor Labs, Cold Spring Harbor, NY.

We sought to examine the possibility that there is a structural correlate to Hebb's hypothesis. A high degree of dynamism, or addition and retraction of branches, occurs in retinorecipient axons in the developing frog retinal ganglion cells (RGCs). When a RGC is first generated by the ventricular progenitor, it soon generates an off-center axon which grows toward the tectal center. If the axon reaches the tectal center, it will branch and rebranch. The branches of these axons will subsequently retract and be replaced with new branches. We used an improved whole-cell patch-clamp technique to record from these axons during development.

We examined the role of NMDA receptor activation (a branch stability) by using APV to block NMDA receptor activation, and the role of neurotransmitter-mediated stimulation in the growth and stabilization of the axons. We found that the axons can be inhibited by muscarinic agonists or glutamate receptor antagonists. We also found that the axons are sensitive to the local environment and that the axons can be stimulated by the introduction of an electrical field. This suggests that the axons are sensitive to their local environment and that the axons can be stimulated by electrical fields.

605.4

INHIBITION OF NITRIC OXIDE SYNTHASE DISRUPTS ON/OFF SUBLIMATION IN THE FERRET LATERAL GENICULATE NUCLEUS. K. K. Craner* and M. Sig. Department of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Retinogeniculate axons in the ferret segregate into eye-specific layers during the first two postnatal weeks. Axons from on-center or off-center retinal ganglion cells further segregate into retinotopically ordered groups by four postnatal weeks in a process dependent upon presynaptic NMDA receptors (Hudspeth et al., Nature 351: 568, 1991). During this period, the lateral geniculate nucleus (LGN) of the ferret transiently expresses NADPH-diaphorase, a nitric oxide synthase (Smith et al., Proc. Natl. Acad. Sci. USA. 90: 891, 1993). Nitric oxide may act downstream of the NMDA receptor as a retrograde messenger during hippocampal synaptic plasticity (e.g., Schwartz and Madison, Science 264: 1891, 1994).

To investigate whether nitric oxide has an analogous role in the development of the on/off sublimation, we blocked nitric oxide synthase during the third and fourth postnatal weeks using an agonist that inhibits the enzyme at the arginine binding site. Ferrets received daily intraperitoneal injections of N-nitro-L-arginine methyl ester (D-NAME) and L-NAME. L-NAME treatment resulted in reduced NADPH-diaphorase staining compared to controls or normal ferrets. Retinogeniculate sublimation was assessed using intracranial injections of WGA-HRP at postsynaptic NMDA receptors. The treatment suppressed the development of the on/off sublimation as compared to controls or normal ferrets. These results suggest that nitric oxide is involved in the segregation of retinal afferents into on/off sublimination in the LGN.

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SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
605.5
RAPID ACQUISITION OF DENDRITIC SPINES BY VISUAL THALAMIC NEURONS AFTER BLOCKADE OF NMDA RECEPTORS. M. Rocha* and M. Sur, Department of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139; and Institute of Biophysics & Neurosciences, Univ. de Sao Paulo, 05508, Brazil.

The NMDA subtype of glutamate receptor plays a critical role in the development of retinogeniculate afferents and postsynaptic target cells in the ferret lateral geniculate nucleus (LGN) (Harsanyi et al., 1991; Rocha and Sur, 1991). Here, we have examined the time course of dendritic changes of LGN cells in vitro after NMDA receptor blockade. Horizontal slices containing LGN somata were obtained from 28 ferret kits between postnatal day 5 (P5) and 45, and maintained alive in a slice chamber. LGN cell dendrites were labeled by DI crystals placed into the LGN, and imaged online employing laser confocal microscopy. Segments of dendrites (n=318) were imaged again after 2 and up to 7 hours of perfusion with control solution, d-APV (50-100 µM), or NMDA (10-100 µM). At P5-6, dendrites often gave rise to short side branches in control and treated slices. Acquisition of dendritic spines occurred after P7 and increased following d-APV perfusion (p<0.05). From P14 to P20, blockade of NMDA receptors resulted in a five-fold net increase in spine acquisition (p<0.001). Changes in spine acquisition declined after P21, and were unaffected by d-APV perfusion. NMDA treatment had no significant effect when compared to control conditions at all ages. Thus, NMDA receptor-regulate dendritic spine acquisition in LGN cells mainly during the third postnatal week, a period when retinogeniculate axons segregate into on and off-center sublaminae. These results may reflect the percentage structural changes in postsynaptic cells contributed to the formation of specific retinogeniculate connections.

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605.7
POSTNATAL DEVELOPMENT OF NMDA RECEPTOR-MEDIATED, NITRIC OXIDE DEPENDENT RELEASE OF NEUROTANIN IMMUNOSTAINING IN VISUAL CORTEX. S. M. Duda, F. W. Hester*, and M. J. Friedlander, Neurobiology Research Center, Univ. of Alabama at Birmingham, AL 35294 USA.

Depolarization or activation of NMDA receptors in cortical synaptosomes preparation from newborn rat brain causes a calcium dependent release of glutamate and neuropeptide which in the case of NMDA receptor activation requires nitric oxide production (Montague, Gacanico, Wynn and Friedlander, Science, 263, 973-977, 1994). More recently, activation of synaptic potentials in adult cortex requires NMDA receptor activation, although in neurons it is independent of NMDA receptor activation (Harsanyi and Friedlander, Soc. Neurosci. Abstr, 23, 1993). Thus, we evaluated the effect on synaptic NMDA receptor-mediated release of neuropeptiain cortex. Cortical synaptosomes were prepared from guinea pigs at various postnatal ages (0.5, 1, 10, 15, 30, 15, and 70 days, n=3, 60, 60, 60, 60, 60, at each age). Release of endogenous glutamate was measured with a luminometric assay and release of exogenous [3H]neuropeptide was measured with scintillation counting. Synaptosomes were counted using the HEPES buffered saline (PBS) and stimulated with either a 50µM potassium depolarizing stimulus or 100µM NMDA either with or without a 30 minute pre-incubation with the nitric oxide synthase (NOS) blocker, L-Nitro-Arginine. Release were expressed as the percent of available transmitter and normalized to the PBS controls. Calcium-dependent depolarization-induced release of both glutamate and neuropeptine remained constant and independent of NOS activity at all ages. However, NMDA receptor-mediated release of both neurotransmitters did not occur until 10-15 days of age, with glutamate release showing a second increase in response between 30 and 70 days of age. At all ages when NMDA receptor-mediated release occurred, it was completely inhibited by NOS blockade. These results suggest that the NMDA receptor/NOS system is immature in the neonate, in agreement with our neurophysiological studies.

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605.8
A CRITICAL PERIOD FOR INDUCTION OF LONG-TERM DEPRESSION IN VISUAL CORTEX IS NEURONTAN INDEPENDENT. S. M. Duda, F. W. Hester*, and M. J. Friedlander, Neurobiology Research Center, Univ. of Alabama at Birmingham, AL 35294 USA.

Activity-dependent weakening of excitatory synaptic transmission has been hypothesized to play a dominant role in the mechanisms that mediate ocular dominance plasticity in the developing visual system. Growing evidence has shown that neuronal activity is critical for various forms of synaptic plasticity, both long-term depression (LTD) and long-term potentiation (LTP). However, it is not clear what role synaptic activity plays in the development of LTD and/or LTP. We have investigated the relationship between synaptic activity and LTD in mature visual cortex. We have shown previously (Duda & Friedlander, Soc. Neurosci. Abstract, 1994) that LTD could be induced in a subset of immature L4 neurons in visual cortical neurons. However our efforts to characterize the developmental expression of this effect have been hampered by the prevalence of IPSP-like hyperpolarizing potentials that contaminate the excitatory postsynaptic potentials (EPSPs); LTD is not induced in neurons exhibiting these hyperpolarizing responses. We have extended our previous findings to include experiments where we have attempted to overcome the effects of this synaptic inhibition. Conventional intracellular recordings were made from layer IV of slices from mature (45 days old) and immature (<8 days old) guinea pig visual cortex and cat (day 1 through adult) striate cortex. In some cases, a mixture of the selective blocker of chloride channels, 4,4-dinitro-2,2-dimino-6-furazinonic acid (DNDS), cesium acetate, and/or bicuculline was included in the electrode. While blockade of inhibition appears to restore the ability to induce LTD in immature animals, it has no effect on the (lack of) induction of LTD in layer IV neurons from adults. These data suggest that while inhibitory synaptic potentials can influence the induction of LTD, their presence does not likely represent a subset of neurons incapable of supporting LTD.

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605.9
INDUCTION OF SYNAPTIC POTENTIATION IN NEONATAL VISUAL CORTEX DOES NOT REQUIRE TYPICAL POSTSYNAPTIC TRICHLOROACETIC ACID ACTION POTENTIALS, NMDA RECEPTOR ACTIVATION OR NITRIC OXIDE PRODUCTION. K. Harsanyi* and M. J. Friedlander, Neurobiology Research Center, Univ. of Alabama at Birmingham, AL 35294 USA.

Low frequency pairings of postsynaptic depolarizing pulses with presynaptic activation causes potentiation of postsynaptic potentials (PSPs) in mature visual cortex (Fregnac, Smith, Burke and Friedlander, J. Neurophysiol, 66, 1025-17, 1991). This type of synaptic plasticity is more reliably induced in the neonate (85% of cells vs. 40% of cells - Harsanyi and Friedlander - Soc. Neurosci. Abstr, 27, 24, 1993). In the present study we determined the mechanism underlying the reliable induction of this form of plasticity in the neonate. Intracellular recordings were obtained from supragranular receptive field sites of layer IV neurons in guinea pig visual cortex (from 5 to 60 postnatal days). Low frequency (0.1 Hz) low intensity (<30% threshold) stimuli were applied to cortical layer 6 with intracellular application of depolarizing pulses (~2 mV, 80 ms, 60 per pairings). In the youngest animals (5-28 days), 85% (n=24/46) of such pairings led to synaptic potentiation (>30% above the pre-potential level after 10 minutes if the former pairing). Application of the NMDA receptor blocker, D-APV (100 µM) did not block induction of synaptic potentiation in this age group (n=11/11). The depolarization-inducing synaptic potentials are effective at inducing synaptic potentiation even during action potential blockade by intracellular application of QX 314, the fast sodium current blocker. Application of the nitric oxide synthase (NOS) blocker, L-Nitro-Arginine at 100 µM for 60-120 minutes did not block synaptic potentiation (n=11/14). Thus, LTD, LTP, and synaptic plasticity in vitro are mediated by postsynaptic receptors, without NO production is sufficient to induce potentiation in the neonatal visual cortex.

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605.10
REDUCED SYNAPTIC PLASTICITY IN VISUAL CORTEX SLICES OF α-CAM-KII KNOCKOUT TRANSGENIC MICE. A. Kidonissi*, A. Silva and M. B. Bear, Department of Neuroscience, Brown University, Providence, RI 02912 and CTR for Learning and Memory, Cold Spring Harbor Lab., Cold Spring Harbor, NY 11724 USA.

Recent work indicates that synaptic plasticity induced in vitro in the superficial layers of visual cortex ocxam camera share remarkably similar mechanisms of induction. Several lines of evidence have implicated calcium-calmodulin kinase II (Cam-KII) in the induction of long-term potentiation (LTP). In previous studies, it was shown that this type of plasticity is induced both by high intensity depolarizing current and by transgenic mouse line that lacks the α-subunit of CAM-KII to address the possible role of Cam-KII in the induction of LTP in the visual cortex. Synaptic responses to stimulation of layer IV were recorded extracellularly in layer III. To induce LTP we used theta burst stimulation (TBS) which previously work has shown to be the most effective method of inducing LTP in the visual cortex. LTP was measured as the increase in field potential amplitude 20 min after TBS. When TBS was applied to slices from wild type animals it resulted in significant potentiation (2.1 ± 0.4%, 34 slices from 6 mice). When TBS was applied to slices from mutants, TBS resulted in little or no potentiation (2.3 ± 1.0%, 44 slices from 7 mice). Furthermore, potentiation of 2% was observed after TBS in 50% of the control cases, 2% LTP was observed in only 5% of the mutant cases.

The dramatically reduced synaptic plasticity in mutant visual cortex to sustain LTP strongly suggests that in the adult neocortex, like in the CA1 region of hippocampus, Cam-KII is crucial for the formation of LTP. Work in progress is aimed at examining (1) if there is a deficit in LTD in the mutant visual cortex, and (2) whether the deficit in LTP applies to the immature as well as the adult visual cortex.
605.11 REGULATION OF LONG-TERM POTENTIATION IN VISUAL CORTEX BY AGE AND VISUAL EXPERIENCE. M.F. Brest and A. Kirkwood, Department of Neuroscience, Psychology and Behavioural Science, Cornell University, Ithaca, NY.

Binocular connections in the visual cortex are modified by sensory experience, but only during a "critical period" of early postnatal life. These modifications may employ mechanisms specific to the visual system, to be distinguished from the long-term synaptic potentiation (LTP) in slices of visual cortex. LTP of synaptic responses in layer III can be reliably elicited by stimulation of the visual cortex of adult animals. However, and we others have found that (in the absence of GABA antagonists) LTP can be induced by white matter (WM) stimulation only in slices prepared from postnatal day (P) 15 rat visual cortex. In the corticocollicular projection has been established. The distribution of D1-labelled corticocollicular axons in the SC was then examined through development.

The corticocollicular axons of neonatally enucleated animals initially extended caudally, past the topographically-generated axons of visual experience. Slices of visual cortex were prepared from 4-5-week-old Sprague-Dawley rats that had been reared either in complete darkness or in a standard lighted environment. Synaptic responses to WM and layer IV stimulation were recorded extracellularly in layer III. In slices from control rats, TBS of the WM had little effect (5.9 ± 3.0% increase in field potential amplitude after 20 min. after conditioning, n = 7 rats), while TBS of layer IV induced substantial LTP (6.6 ± 6.6%, n = 12 rats).

In contrast, slices from dark-reared rats LTP was evoked equally well by TBS of the WM (6.0 ± 3.7% in layer IV; 7.4 ± 4.8% in cells, respectively). The present results indicate that the history of visual experience can be evaluated by a system that allows for the study of the effects of visual deprivation on the development of the visual system. The corticocollicular projection is dependent upon normal visual function.

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606.1 NOVEL ENHANCER TRAP MUTANT REVEALS DIVERSITY OF OLFACTORY FUNCTION IN DROPSOPHILA MELANOGASTER. Adrienne E. Dubin*, John Molina, Halsh Razagzari, Brett Ferson & Greg Harris. Brain Imaging Unit, State University of New York at Stony Brook, Stony Brook, NY.

Similarities between olfactory systems in vertebrates and insects suggest that the general rules underlying olfaction are conserved. We have taken a genetic approach to identify genes involved in olfaction in Drosophila. Enhancer trap line HD1 expresses the reporter gene lacZ encoded by a P-element inserted near position 64 in a subset of cells in the antennal gland (the main olfactory organ). HD1 flies have no detectable olfactory defects, however, after excision of the transposon, at least 2 of 6 independent lines revealed a dominant impaired sense of smell to the potent odorant ethyl acetate (EAC) as assessed in a behavioral assay and electroantennogram recordings. The percentage of excision line 15A flies different to EAC in the behavioral assay (17.5%) (mean ± S.D, n = 7) was significantly higher than controls (68.5%) (mean ± S.D, n = 7; p < 0.005). EAC acted at 10-3 dilution for 500 ms from a cotton-plugged puff pipe pipet caused a 4-fold decreased response in control flies (15A (1.75±2.62 mV, n = 13); compared to HD1 (6.9±0.70 mV, n = 17; p<0.005) and another excision line (15B: 7.6±0.5 mV, n = 18; p<0.005). Responses to benzaldehyde, propionate, butanol, isobutanol, acetate, acetic acid were unaffected. The maximum response to EAC was decreased with no change in the EC50. Responses to short aliphatic chain acetates were most affected (compared to controls): 25% (EAC), 41% (PropylAC), 54% (EthylAC), 100% (ButylAC and tert-Butylacetate AC). A molecular analysis of the mutation is underway. Supported by AHA, California Affiliate 92-275 and NSF BNS-9022212.

606.2 HETEROLOGOUS EXPRESSION OF AMILORIDE-SENSITIVE SODIUM CHANNEL SUBUNITs AND AN ALTERNATIVELY SPICED FORM IN TASTE TISSUES. A.J. Li and S.H. Snyder. Dept. of Neurochemistry, Johns Hopkins Medical School, Baltimore, MD 21205.

The transduction of sodium salt taste is mediated by voltage-independent, amiloride-blockable sodium channels (ASCI) in taste cells. Recent molecular cloning studies have revealed that an amiloride-sensitive epithelial sodium channel isolated from rat colon consists of three homologous subunits (α, β and γ) (Catenzela et al Nature 367, 463-494, 1994). Utilizing a polymerase chain reaction and Northern blot analysis, we have identified the presence of all three subunits and an alternatively spliced form of γ subunit of ASC in rat tongue epithelial tissues that contain taste receptor cells. The heterologous expression of α, β and γ subunits in taste tissues appear to be distinct from that in lung and kidney. In lung and kidney, however, the expression levels of β and γ subunits are about same whereas the expression of the α-subunit is the greatest. The expression levels and tissue distribution of the alternatively spliced form of the γ-subunit of ASC are similar to that for the native α-subunit: higher in lung and kidney and lower in taste tissues. Preliminary studies on transfected cells expressing ASC γNAs showed that α and β subunits, but not γ subunits, were able to bind [3H]-phenamil, an amiloride analog. The heterologous co-expression of ASC α-subunits may contribute to tissue specific functions of ASC such as salty taste.


We have previously shown that vertebrate olfactory receptor cells have narrow dynamic range responses to odours. Does response envelope studied in the presence of different concentrations of cisnol, isonitryl acetate or acetophenone for an exposure time of 1.2 sec were well fitted by the Hill equation with a Hill coefficient of between 1 and 1.5 times values between 3 and 100 pM. Although the Hill coefficient for these odorants was in the micromolar range, the cellular threshold for odorant detection could be significantly lower. We have investigated this possibility we exposed a single cell to low odorant concentrations for longer periods of time. In a cell with a K1/2 of 10 µM as measured from dose-response curves of odorants isolated current bumps induced by 500 nM of the same odorant. These bumps had a bell shape with amplitudes of 1-10 pA and a duration of 300-500 ms. Olfactory receptor concentrations lower than previously shown. They may accomplish this by sampling over longer periods and integrating information over time. Thus a cell expressing a bromide tuned low affinity odor receptor may recognize sensitivity at the expense of temporal resolution, as apparently sensitive trade-off for olfactory neurons. The observed small current bumps could be caused by the binding of a single odor molecule to a receptor, as in photoreceptors it is possible to observe quantum bumps caused by the absorption of a single photon. Supported by EEC BSS 9651, NIH, ONR and NATO.

606.4 SALAMANDER OLFACTORY RECEPTOR NEURON ODOR RESPONSES DEPEND UPON STATES ESTABLISHED BY PRIOR STIMULUS PRESENTATIONS. R.C. Gesteland*, P. Farmer and M. Overwijk. University of Cincinnati Medical Center, Cincinnati, OH 45267-0521.

Voltage-sensitive dyes observed with a confocal microscope allow the activity of each cell to be observed on a time course. Odorous stimuli were found to be different among groups of cells, and that some groups were more sensitive to the same odors than others. However, it is likely that these differences in the direction of action can be long-lasting. The response of a cell to a stimulus will depend upon the activation states of the various channel types resulting from prior odor experience. This work was supported by NIH grants DC00342 and DC00347.
606.5
OLFACTORY TRANSDUCTION IS INTRINSICALLY NOISY. Graeme Lowe & Geoffrey H. Gold. Monell Chemical Senses Center, Philadelphia, PA.

The high level of noise in olfaction suggests that olfactory receptor cells reliably detect single odorant molecules. Discrete events, presumably triggered by single odorant molecules, have been observed in insect pheromone receptor cells, but analogous events have not been observed in vertebrate systems. We have sought to resolve single molecular responses in rat by recording the transduction current in dissociated olfactory receptor cells. When cells were exposed to threshold concentrations of one of the odorants, methamphetamine, 2-isobutyl-3-isopropylpyrazine, isopropyl acetate or 2-ethylpyridine, the whole-cell current displayed pronounced low frequency (< 10 Hz) fluctuations which might represent the summation of single molecular responses. However, fluctuations of similar amplitude and frequency spectrum were also observed in the absence of odorants by inhibiting basal phosphodiesterase activity with IBMX, or by photolytic release of cyclic AMP. Thus, the odorant-induced fluctuations represent the basal, or intrinsic, noise of olfactory transduction, not the activation of receptor proteins by odorants. In unstimulated cells this noise is suppressed by the threshold inherent in cyclic-AMP activated current. Our data suggest that for many common odorants, single odorant molecules are not reliably detected by mammalian olfactory receptor cells.

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606.7

Mr and CuZn superoxide dismutases (SODs) are enzymatic scavengers of superoxide free radicals. Mitochondrial MrSOD and cytosolic CuZnSOD were localized immunohistochemically in olfactory mucosa obtained at autopsy from 19 subjects, 11 males and 8 females, ranging from 24 to 98 years of age. Four patients were classified as young/middle aged (under 60 years old), 6 as old (over 60 years old), 4 (69-98 years old) as "old", and 5 (72-90 years old) as "old/adult". Histologically confirmed Alzheimer's disease (AD). Postmortem intervals (PMIs) ranged from 2-22 h. In young/middle aged subjects, intense immunoreactivity was observed in olfactory receptor neurons, sustentacular and basal cells in the olfactory epithelium and in Bowman's glands, olfactory nerves, and vascular endothelium in the lamina propria. Comparing young/middle aged and old, there was an age-related decrement in the overall intensity and distribution of immunoreactivity that was most pronounced in olfactory receptor neurons, basal cells, and endothelium. Old subjects often had restricted (oc) of intense epithelial immunoreactivity. In the "successfully aged," large regions of immunostaining were extensive as that in young/middle aged subjects were intermingled with smaller regions of reduced intensity. Subjects with AD generally exhibited the most intense staining that was distributed like that in the "successfully aged." These differences were not related to PMI; the 3 youngest subjects had the longest PMIs. 3 old subjects had short PMIs; age- and AD-related trends were observed in subjects from the different groups matched for age and PMI. High levels of SODs in the young/middle aged and "successfully aged" may reflect the higher cellular viability of their olfactory mucosae, while high levels in the AD subjects may be related to oxidative stress associated with the disease process. Supported by NIH grants DC 01175 (MLG) and DC 00159 (TVG).

606.8

In the turtle olfactory bulb, primary olfactory neurons are thought to excite mininaluflbach (MT) cells by stimulating both NMDA and non-NMDA glutamate receptors (Berkovics et al., J. Neurophysiol. 71, 1994). It has been suggested that the non-NMDA receptors allow MT cells to respond rapidly to incoming odor information while the NMDA receptors might provide a means for the short-term temporal integration of this information. We have examined this idea by optically monitoring electrical activity in the isolated turtle olfactory bulb stimulated with the voltage-sensitive dye, RH 155. The figure below shows optical responses recorded from the external plexiform layer evoked by paired-pulse stimulation (500 ms separation) of the olfactory nerve before (thin trace) and after (thick trace) addition of the NMDA antagonist, AP-5 (100 µM), to the bathing solution. As can be seen, NMDA blockade partially reverses depression of the second (test) response but has little effect on the initial (conditioning) response. Supported by NIH GM07517.

606.9

The olfactory system is a remarkable model to study in vivo basic phenomena occurring in neurones of the CNS. Olfactory neurones in the olfactory mucosa send their axons to the glomerular layer of the olfactory bulb where they make synapses with several neuronal types. An important consequence of peripheral afferent denervation is the olfactory bulb atrophy reduction of neurotransmitter dopamine due to loss of tyrosine hydroxylase (TH) mRNA. In the present study we began to address the question of whether the expression of subunits belonging to various GABA- and glutamate receptor complexes is modified in unilateral chemical lesion (ZnSO_4-irradiation) of the rat olfactory mucosa. Analysis of the olfactory receptor total RNA was performed on 3 groups of male adult rats [(i) untreated, (ii) saline- and (iii) ZnSO_4-irradiated rats], sacrificed 12 days post-lesion. Both competitive polymerase chain reaction (PCR) assays and semiquantitative PCR experiments were employed to compare the relative abundance of mRNAs in the 3 groups. Measurements of TH mRNA were used as an indication of the effectiveness of the lesion. In ZnSO_4 irradiated rats we found a remarkable reduction both of the γ_2 and γ_3 GABA receptor subunit mRNA, while we observed only a slight increase in the NMDR1α mRNA splicing form and no significant change in the NMDR1α and in the GluR1 mRNAs. Our findings suggest that the expression of at least some GABA receptor subunits in the olfactory bulb are under transynaptic regulation. Since it has been recently demonstrated that glutamatergic-immunoreactivity is present in olfactory neuron terminals (Sauss-Poggetto et al., Neuroreport, 1993), loss of release of those may be responsible of the decrease of TH mRNA that might be followed by a reduction of transmitter release and modulation in the expression of GABA_α receptor genes in post-synaptic elements.

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606.10
OLFACTORY AND CHEMICAL INJURY OR SENSITIVITY. Donald L. Budney. Chemical Injury Research Foundation, Tacoma, WA 98407.

Auditory, visual and upper and lower somatosensory evoked potentials were studied in 20 patients with clinical criteria for chemical sensitivity. Measures were made before and during exposure to chemicals that volatilized and led to complete debilitation.

In general, auditory and visual P3 latencies and N19, P22, P31 and N45 latencies were significantly increased (p<0.001). In general, amplitude for the same variables was not significantly changed (p>0.20). An exception was noted in the P3 amplitude which was significantly decreased (p<0.001).

Latency correlations were low or not significant prior to exposure and highly significant during exposure. On the other hand, amplitude correlations were highly significant prior to exposure and not significant during exposure. Since olfactory signals are sent to nearly every part of the brain it is thought that this system's use of excitatory amino acids and their precursors for neurotransmission in association with cholinergic cells with six or less carbon fragments that volatilize could lead to brain cell injury and subsequent neuronal changes that are experienced by the patient as the signs and symptoms of chemical sensitivity or injury.

The above will be discussed in association with agonists and antagonists of excitatory amino acids in humans.
A 3-D FUNCTIONAL MRI DEMONSTRATION OF BRAIN FUNCTIONS INVOLVED IN HUMAN OLFACTORY FUNCTION. N.E. Ramsay, B. Kawaguchi, A. Shulman, M. D. LeMay, and S. Hoemmer.* National Institute of Alcoholism and Alcohol Abuse, NIMH in Vivo Center, Laboratory of Diagnostic Radiology Research, NIH, Bethesda, MD 20892.

Olfactory processing selectively involves the limbic system. Imaging of limbic areas with fMRI is problematic, due to their location near the base of the skull, close to blood vessels. We report here an olfactory stimulation study involving a 3-D Echo-Shifted FLASH sequence and a Gaussian random noise baseline for motion correction (Worsley, K. J., Evans, A. C., Marrett, S., et al., J. Cereb. Blood Flow Metab. 12, 900–918, 1992). Pleasant odors were administered to 10 subjects (9a). After ananesthesia, 3T-HASTE stimuli were done at 3 minute intervals. Each trial consisted of two functional scans, with 7 seconds between trials. Olfactory stimulation was switched on at the end of the first scan, and off at the end of the second scan (odor duration 27 secs.). For each subject, data were converted to difference images, obtained by subtracting the unstimulated from the stimulated 3D image within each trial. Trials were then summed, and SA averaged individually. Significantly activated voxels were found in structures of the olfactory system, including the amygdaloid complex (556), septal nuclei (556), and ventromedial striatum (556), posterior orbital cortex (45). Very few significant voxels were found outside of the olfactory system. These results indicate that the 3D-Echo Shifted Flash sequence enables in vivo imaging of olfactory function.

607.2 TALIN FUNCTION IS SPATIALLY INTEGRATED IN THE NEURONAL GROWTH CONE. Anne M. Snyder* and Daniel C. Jay, Department of Cellular and Molecular Biology, Harvard University, Cambridge MA, 02138.

Dynamic extension and retraction of filopodia is thought to function in signal detection and force generation needed for directed motility of the neuronal growth cone. To address the role of cytoskeletal proteins in filopodial motility we have used micro-CALI (Chromophore Assisted Laser Inactivation) to functionally inactivate the cytoskeletal protein talin in growth cones of chick DRG neurons in culture. We have found that inactivation of talin in a region of the growth cone results in a loss of filopodial extension and retraction, whereas inactivation of talin over the whole growth cone has little effect on filopodial motility. Micro-CALI inactivates proteins within a 10-15 micron spot by directing the energy of a 620 nm laser beam to the protein via a dye-labeled antibody. Neurons were trypsinized, rinsed, and reacted with green-labeled antibodies to talin and regions of the growth cone were laser irradiated for five minutes. This caused the rate of extension and retraction of filopodia within the irradiated area to drop to zero for one to two minutes; filopodia outside this region showed no change in motility. Irradiation of whole growth cones at the same laser power did not result in any change in filopodial behavior. Laser irradiation of malachite green-BSA loaded controls showed no change in growth cone motility. This evidence that talin can act to couple filopodial movement to cytoskeletal dynamics. These data also suggest that the growth cone spatially integrates internal differences in protein function. We believe such spatial integration of protein function could provide the basis for directed growth cone motility and are investigating this possibility.

607.4 NERVOUS SYSTEM ABNORMALITIES RESULT FROM TARGETED MUTATION OF MAP2B. W. Edelman, P. Costello, P. Davies, L. Roback, B. H. Walner*, R. Kucherlapati, Deps. of Molecular Genetics, Neurosurgery and Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

For proper development of the brain, an intact complement of cytoskeletal elements is essential, including that of the microtubule associated proteins. The microtubule associated protein 1b (MAP2B) is a member of this microtubule network in neurons and glia. MAP2B expression is developmentally regulated and is highest during embryogenesis and the early postnatal period. This expression pattern suggests involvement in the regulation of neurite outgrowth. We have targeted MAP2B in the MAP2B gene which resulted in embryonic lethality in homozygous mice and a marked phenotype in some heterozygous animals. This phenotype includes hindlimb hypoplasia, a spastic tremor of the hindlimbs and an overall reduction in body weight of 25-50%. Histologically, the phenotype is pronounced in the cerebellum. The principal abnormality is a dramatic reduction in the Purkinje cell bodies and their dendritic arborizations. Immunohistochemical studies with MAP1B and MAP2B specific antibodies showed reduced staining in Purkinje cells. No decrease in staining of another cytoskeletal protein, MAP2, was observed. Western blots revealed no change in levels of neurofilament proteins, MAP2 or tau. A differential abnormalities were seen in the hippocampus, olfactory bulbs and visual system. These data suggest an essential role of MAP2B in the development of the brain.
607.5 EVIDENCE THAT CAMs STIMULATE NEURITE OUTGROWTH BY ACTIVATING FGF RECEPTORS IN NEURONS. P. Dobony*, E.J. Williams, J. Furness and F.S. Walsh. Department of Experimental Pathology, University of Cambridge, London SE1 8RT.

We have used monolayers of parental 3T3 and 3T3 cells expressing one of three transfected cell adhesion molecules (CAMs) (ICAM, N-cadherin and L1) as a culture substrate for rat cerebellar neurons. A number of lines of evidence suggest that neurite outgrowth stimulated by the above CAMs involves activation of the FGF receptor in neurons. After suggesting that the CAMs which bind to the FGF receptor in neurons inhibit neurite outgrowth stimulated by the above CAMs but have no effect on integrin dependent neurite outgrowth or neurite outgrowth stimulated by a variety of other agents. In addition we have found that a soluble L1-Fc chimera is effective as cell associated L1 in promoting neurite outgrowth. The response to the L1-Fc chimera is tuck correlated with the presence of CAMs. This suggests that the CAMs which bind to the FGF receptor with basic FGF. We conclude that activation of the FGF receptor, rather than changes in adhesion, underly the neurite outgrowth response to a number of CAMs.

607.6 PERMISSIVE ROLE OF GAP-43 IN NEURITE OUTGROWTH: PRIMARY SENSORY NEURONS AND TRANSGENIC MICE. L. Aigner, F. Botteri, and P. Caroni*, Friedrich Miescher-Institute, P.O. Box 2543, CH-4002 Basell, Switzerland.

The specific association of the growth-associated protein GAP-43 with nerve growth during development and regeneration suggests that it may play a role in this process. Early studies (Schnell et al., Neurosci. Rev. Neurosci. 12:1227). Downregulation of neuronal GAP-43 expression during development coincides with the process of synapse elimination. Suggesting that it may also play a role in local process outgrowth and maintenance. To define the role of GAP-43 in neurite outgrowth we have 1) analyzed neurite and growth cone (GC) activity in primary sensory neurons specifically depleted of (GAP-43) by an antisense approach (Aigner and Caroni (1993) JCB123:417), and 2) generated transgenic mice that constitutively express chick GAP-43 in adult neurons. Characteristic features of neuronal antibodies that bind to L1 or the FGF receptor. The response is also associated with an increase in phosphotyrosine on the same set of neuronal proteins. We propose that this increase is a consequence of the tyrosine phosphorylation of the FGF receptor with basic FGF. We conclude that activation of the FGF receptor, rather than changes in adhesion, underly the neurite outgrowth response to a number of CAMs.

607.7 GAP-43 IMMUNOREACTIVITY AND AXONAL REGENERATION OF RAT RETINAL GANGLION CELLS. H. Schaden*, M. Bihr*, C.A.O. Steumer, University of Konstanz, Germany, *MPI Tübingen, Germany.

While more than 90% of the retinal ganglion cells (RGCs) in rats die upon optic nerve section (ONS), a subfraction of those that survive is capable ("competent") of regenerating an axon into a peripheral nerve (PN) graft (Yan et al., 1987). To determine whether re-expression of competent RGCs, the relevant antibodies were applied to sections of retina (i) in rats with acute ONS only (ii) in rats which had received a PN graft. RGCs were labeled by the retrograde tracer Fluorogold (FG) applied to (i) the ON to identify surviving RGCs, (ii) to the PN graft to identify RGCs with regenerating axon (iii) GAP-43 immunoreactivity (i) was seen in 22% of RGCs at 5d after ONS and in 2% and 1% at 14 and 21d, respectively, in rats with out grafts. (ii) In rats with PN grafts, only those RGCs which were FG-labeled (and thus had regenerated an axon into the graft) exhibited GAP-43, and they represented 3% and 2% at 21 and 28d after surgery, respectively. c-JUN, however, was found in (i) many more RGCs than GAP-43 and in (ii) at least twice as many RGCs at 21 and 28d, than were there RGCs labeled with FG and exhibiting GAP-43-ir.

Thus regenerating axons in PN grafts derive specifically from GAP-43 re-expressing RGCs. Appearance of GAP-43 may therefore identify those RGCs that possess the intrinsic property of axonal regeneration or sprouting.

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607.8 REDUCED PRESYNAPTIC ACTIVITY DURING NEUROMUSCULAR DEVELOPMENT PROMOTES FOREIGN MOTOR ENDINGS IN DROSOPHILA. J. Jacob*, and I. Kaschhian, Genetics and Biology Dept., Yale Univ., New Haven, CT 06511.

Electrical activity plays a role in the development of Drosophila neuromuscular synapses. We previously showed that blocking presynaptic (but not postsynaptic) activity during embryonic synaptogenesis promoted inappropriate motoneuron contacts (Liu & Hom, Neurosci. Lett., 1965). In order to follow the development of these ectopic contacts during larval life, the activity of the muscle afferents arising from the nerve cord was examined (paral*, nap*, and so*). Collateral sprouting increased into muscle fibers 65% in the mutant embryos when reared at 34°C during stages 14-18. (WT: 16% sprouts, n=169 segments; paral: 25%, n=150, p<0.05; nap*: 29%, n=91, p<0.005; paral* vs. nap*: p<0.005; n=103, p<0.05). An increased axonal outgrowth per unit area of muscle endings was also observed in these fibers in nap* and so* but not paral. (WT: 5.6% of fibers receiving sprouts, n=89 segments; paral: 8%, n=110; paral*: 10%; p<0.005; paral* vs. so*: p<0.005; paral vs. so*: p=0.05). About 60% of the ectopic endings arise from the transverse nerve, 50% from endings on the oblique muscle fibers, and less than 5% from endings on muscle fibers 13 or 8. The morphology of the native neuromuscular endings is not changed by the presence of the ectopic synapses. Temperature shifting (37°C-25°C) during larval development further increased the number of ectopic endings to 50% in nap* and 34% in so* (n=164) as opposed to shifted WT animals (6%, n=62) and shifted paral* (2.4%, n=60). Significant increases were not observed in hyperactive mutants such as eag sh or so*. The critical period includes both late embryogenesis and the 1st instar. We hypothesize a reduction in synaptic activity in the embry发育 induced filodipal sprouting, and that in the 1st instar reduced synaptic activity stabilizes them. These results show that synaptic activity contributes to the maintenance of correct neural connectivity.


In the adult rat, acute treatment with kainic acid, a glutamate agonist, induces FGFR-43 mRNA in granule cells maximally at 24 hr (McNamara et al., Soc. Neurosci., 1993). KA also induces sprouting of granule cell axons, the mossy fibers, into the supragranular layer beginning ~10 hr after KA (Nadel et al., Brain Res., 182:1, 1980). To induce the rotation of FGFR-43 mRNA in granule cells to subserve mossy fiber sprouting, adult male Sprague-Dawley rats were injected with: 1) saline 2) KA (i.e., 10 mg/kg), 3) PENT (i.e., 30 mg/kg) 4) PENT then KA 15 min later, 5) MK-801 (i.e., 2 mg/kg), and 6) MK-801 then KA 15 min later. Rats were sacrificed either (A) 24 hr after the last injection and FGFR-43 gene expression assessed using quantitative in situ hybridization or (B) 30 d later when mossy fiber sprouting was assessed with Timm's stain. KA alone first induced FGFR-43 mRNA in granule cells as well as subsequent mossy fiber sprouting into the supragranular region. Visual inspection revealed no significant cell loss in areas CA3A/CA4A after KA alone. Pre-treatment with PENT of the KA-induced FGFR-43 mRNA induction and supragranular sprouting but, except in one case, did not affect cell loss in CA3A/CA4A. As shown by in situ hybridization, MK-801, only minor mossy fiber sprouting was observed. When administered alone, PENT and MK-801 did not affect FGFR-43 mRNA, mossy fiber sprouting, or cell survival. In conclusion, kainate receptor activation leads to mossy fiber sprouting. This sprouting appears to be independent of excessive cell loss in areas CA3A, supported by MO58253 to A.R. and Post-doctoral fellowships from NSERC to K.R.M. and NSF to P.A.J.

607.10 INCREASED SPROUTING OF PRIMARY AFFERENTS IN THE MYELIN-FREE RAT SPINAL CORD. Joseph P. Kuphaner*, Guido Schweger, and Martin E. Schwab, Brain Research Institute, University of Zürich, Augustin-Foehr-Str. 1, 8029 Zürich, Switzerland.

We are interested in defining physiological functions of myelin-associated neurite growth inhibitors. These molecules are involved in the prevention of axonal regeneration in the adult mammalian CNS (Schnell et al., Nature 367:170, 1994). Previous work has shown that the regional expression of GAP-43, a putative marker for fiber growth and plasticity, is inversely related to the degree of myelination in the CNS of normal rats (Kaphammer and Schwab, JCN 340:194, 1994). Furthermore, GAP-43 expression is strongly increased in myelin-free lumbar spinal cords when myelinated motor terminals are ablated by neonatal X-irradiation (Kaphammer and Schwab, Eur.J.Neurosci. 6:403, 1994). These results suggest a role for the inhibitors in re-inhibiting sprouting and anatomical plasticity in the normal CNS.

We have now investigated whether the increased expression of GAP-43 in the myelin-free spinal cord is indeed correlated with an increased potential for sprouting fiber terminals. Nerve sprouting from the roots of lumbar segments L2-L4 were cut in myelin-free and normal cords of 8 or 15 day old rats. Sprouting of primary afferents was studied 3 weeks later when the threementioned motor terminals were myelinated by the neonatal X-irradiation. This enzyme specifically labels a subclass of spinal cord primary afferents. We found that the sprouting of TMP positive afferents is increased in the myelin-free condition as well as in the irradiated cord. This further supports our hypothesis that CNS myelin and its associated neurite growth inhibitors are involved in the regulation of terminal sprouting and plasticity in the normal CNS.

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608.1 RULES DETERMINING THE MONOSYNAPTIC CONNECTIONS BETWEEN LGN CELLS AND SIMPLE CELLS IN CAT VISUAL CORTEX. R. Clay Reid, Jose-Manuel Alonso and Torsten N. Wiesel. Laboratory of Neurophysiology, The Rockefeller University, New York, NY.

We have studied the rules that determine whether an individual afferent from the lateral geniculate nucleus (LGN) will have a monosynaptic connection with a given cortical simple cell. Our question was: how precisely can the synaptic connections between these two cell types be predicted by their functional properties? Single units were recorded with electrodes in both LGN and cortex. Receptive field position and structure were matched with white noise stimuli. Connectivity between pairs of cells (one LGN cell and one simple cell) were compared with cross-correlation analysis of the spike trains. Narrow peaks with very short latencies were taken as evidence for monosynaptic connections.

The rules of connectivity are very precise. Over 80% of cell pairs were monosynaptically connected when a receptive field was centered over a simple cell subregion of the same sign (on or off). Connections were never seen when an different sign LGN receptive field was centered over a simple subregion (on vs. off). The probability of monosynaptic connection also depended on a good match between the receptive field sizes and the time-course of the responses. In particular, fast LGN afferents were less likely to be connected to a simple cell if the overlapping simple subregion had slower dynamics than the LGN center.

The elongated distributions of the direct on an off LGN afferents to individual simple cells explains at least part of cortical orientation selectivity. It does not, however, reject an important role of intracortical processes in sharpening this tuning.

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608.3 VISUALLY EVOKED CALCIUM ACTION POTENTIALS IN SIMPLE AND COMPLEX CELLS IN THE CAT STRIATE CORTEX. Judith A. Hirsh*, Jose-Manuel Alonso, and R. Clay Reid. Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

Synapses made by thalamic afferents comprise a small minority of those made in layer 4, yet their input largely accounts for the simple receptive field. One means of enhancing excitatory synaptic input is through voltage-dependent calcium conductances in the postsynaptic membrane. We examined the role of these in the construction of visual cortical receptive fields by making whole-cell recordings in vivo.

We mapped receptive fields with randomly flashed bright and dark squares (Jones and Palmer, 1987; J. Neurophysiol. 58: 1187). Intracellular labeling showed that the simple cells we studied were either spiny stellates or pyramidal neurons. When recordings were made with a conventional internal solution, visual stimulation evoked large, fast action potentials. Intracellular blockade of these sodium spikes with QX-314 routinely revealed, on visual stimulation or current injection, smaller, slower action potentials presumably mediated by calcium. Moreover, we could map receptive fields with the visually driven calcium action potentials as we usually do with sodium spikes. Parallel results were obtained for pyramidal cells with complex receptive fields, by whole-cell recording in vitro.

(Supported by NIH EY05893 and EY02113 and a Klingenstein Award to J.A.H., a Fulbright/MEC to J.M.A., NIH EY10115 and a Klingenstein Award to R.C.R. and NIH EY02523 to T.N.Wiesel)

608.2 COUPLING BETWEEN NEIGHBORING LGN CELLS: POSSIBLE IMPLICATIONS FOR SIMPLE RECEPTIVE FIELDS. Jose-Manuel Alonso*, R. Clay Reid. Laboratory of Neurophysiology, The Rockefeller University, New York, NY 10021.

The receptive field of simple cells is constructed largely by the convergence of the aligned input from the lateral geniculate nucleus (LGN). However, extracellular and intracellular interactions could also determine part of the final shape of the receptive field. Here we are presenting evidence for excitatory connections between neighboring LGN cells recorded with the same electrode. Each receptive field was mapped with a pseudo-random dynamic checkerboard stimulus (m-sequence), and the spikes were isolated with the Brainwave System. Forty percent of the LGN pairs studied showed strong and narrow peaks with cross-correlation analysis. This percentage was even larger for those pairs with good overlap of the centers (>20%), and well matched size and timing between the receptive fields of the two cells. Asymmetric narrow peaks were seen mainly when the two cells had partially overlapped on and off receptive fields. Symmetric narrow peaks were more frequent in pairs with partially overlapped either on/on or off/off receptive fields. If, as we suspect from our geniculocortical study, the connected pairs feed into the same simple cell this could provide a means to generate a synergistic input that could drive the simple cell to threshold. (Supported by Fulbright/MEC, NIH EY10115 and EY02523, and the Klingenstein Fund)

608.4 SYNAPTIC RESPONSE PROPERTIES OF COMPLEX CELLS IN THE CAT STRIATE CORTEX. Maria V. Sánchez-Vives*, Judith A. Hirsh, Jose-Manuel Alonso and R. Clay Reid. Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

Complex cells in the superficial layers of area 17 often have more transient responses than the simple cells in layer 4 that provide much of their input. We used whole-cell recording in vivo to measure the relative role of synaptic excitation and inhibition in the generation of these briefer responses. The stimuli were randomly flashed bright and dark squares each lasting 40 ms. Most of the cells we recorded from were pyramids, as identified by intracellular labeling. In these cells, the stimulus evoked EPSPs were frequently followed by marked inhibition. This inhibition seemed large in part to be mediated by chloride, as it persisted in the presence of QX-314 which suppresses the slow IPSPs mediated by potassium. The strong inhibitory component of the response observed in pyramidal cells suggests that there is a population of inhibitory interneurons driven by the rapidly flashed squares. In fact, we have recorded from a large basket cell that was vigorously excited by these stimuli. Such cells project densely to pyramidal neurons (Somogyi et al., 1983, Neuroscience V10: 261). We propose that the inhibition mediated by smooth cells regulates the temporal properties of their target neurons in the superficial layers of the visual cortex. (Supported by F.P.I. (MEC) to M. V.S.V, NIH EY09593 and a Klingenste Award to J.A.H., Fulbright/MEC to J.M.A., NIH EY10115 and a Klingenstein Award to R.C.R. and NIH EY05253 to T.N.Wiesel)
THE NATURE OF INPUTS UNDERLYING SIMPLE CELL DIRECTION SELECTIVITY. D. Fields* and L. Kostyshin Dept. of Neurobiology, Northwestern Univ., Evanston, Ill 60208 and Smith-Kettlewell Eye Research Institute, San Francisco, CA 94115.

Direction selectivity in simple cells of cat area 17 appears to be based on variations within the receptive field of the time course of the response to visual stimuli. Response latency to flash stimuli is longer in those parts of the receptive field first encountered by a stimulus moving in the preferred direction, suggesting that the latency varies smoothly across the receptive field or abruptly in a small number of shifts, and we have characterized the properties of the inputs underlying these variations.

Intracellular recordings from different parts of the receptive field indicated that the potential evoked by stationary sinusoidal gratings at 8 spatial phases (Science 257:1901) were applied to a singular valued decomposition (SVD). This analysis showed that each of the 8 responses (Rn, where n = 8 spatial phase) could be expressed with up to 98% accuracy as a linear combination of 2 functions, f1(x) and f2(x). I.e., Rn = a1 f1 + a2 f2. The coefficients a1 and a2 varied nearly sinusoidally with spatial phase of the stimulus grating, indicating that the two types of synaptic input underlying f1 and f2 were in linear spatial summation. The SVD does not provide the actual input signals from the two types of synaptic input; f1 and f2 are instead linear combinations of those signals. To derive the input signals, a minimization procedure was applied to the even harmonics of Rn. The inputs derived from this procedure were approximately 90° out of phase with one another at the optimal spatial frequency, and delayed with respect to one another by 60 to 80 ms. They were also of unequal strength, the mismatch in strength being greater for cells with lower direction selectivity.

From this analysis we conclude that 1) simple cells receive input from two cell types with different response latencies; 2) the two types are spatially linear but temporally nonlinear; 3) the simple cell sums these inputs in a highly linear fashion.


Neurons in macaque area VI respond more strongly to texture displayed over their receptive fields when that texture appears to be part of a discrete, object-like region of the scene than when the texture appears as part of a homogenous background. Extra-receptive field mechanisms are thought to play a role in scene analysis at a scale larger than their receptive field area. Here we used single and multiple cell recording to study the spatial extent of extra-ness in parietal area VI of the awake monkey. We used texture displays in which a disc-shaped region was delineated from the background by contrast in orientation, brightness, or color. As the monkeys watched, texture displays were presented with the disc region centered on the receptive field of the VI neuron under study; texture over the receptive field was identical in all cases during recording from a cell. The diameter of the disc was varied between 1.8° and 14.4°, always larger than the receptive field region of cells under study. The resulting response profile to texture displays could be seen as an initial burst of activity at texture onset that was similar under all conditions, and a subsequent tonic response period that showed enhanced response for disc versus homogenous texture background. Enhanced response to discs declined monotonically with increasing disc diameter, falling off for discs of diameter 12°, on average. The time course of this modulation generally did not depend on disc size. Experimental controls showed that these effects were not a result of visual attention or small eye movements.

608.9 THE RELATIONSHIP OF RECEPTIVE FIELD COVERAGE TO FUNCTIONAL MODULES IN PRIMATE VI, C. L. Landamart, A. W. Rom, and D. L. Schiller Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

*Rockefeller University, New York, NY. 10012

We are still not sure how the different functional modules in parafocal VI provide coverage of visual space. In particular, how do ocular dominance columns and color patches provide full representations of visual space, given their discontinuous organization in VI? Are the maps continuous or independent across the different modules? Using a combination of tangential electrode penetrations and a series of perpendicular penetrations guided by optical images of functional activity, we examined visual space across ocular dominance columns and across bands. In long tangential penetrations crossing OD columns, we have confirmed the existence of receptive field "jumps" at ocular dominance borders. Within OD columns, we have observed regions, roughly monotonically oriented, within ocular dominance borders, we have observed jumps between left and right eye progressions, some of which are consistent with the two-steps-forward, one-step-back progression described by Blakemore and Wintle.

We are particularly interested in coverage by color in VI. The occurrence of color selective cells in tangential penetrations is much lower than in perpendicular penetrations, in matching color regions reached with linear optical images. In the tangential penetrations, only about 20 percent of cells encountered were unoriented cells and approximately 15 percent were color selective cells. Only 3 to 4 percent were the classic color-opponent unoriented cells. In targeted penetrations, 50% were color-selective cells and 35% were broad band unoriented cells. Thus, by using the optical images to target a row or a region of color-selective patches, color cells can be located with a higher frequency. We are using this technique to study coverage by the color system.

(Supported by grants GM07524, EY02420, ONR N00014-91-J-1865 and McKnight).

608.6 Effect of Eye Mispaligment on Receptive Field Formation in Realistic Visual Environments. Hard Shoval, Nathan Intraorot, C. Charles Law, and Leon N Cooper*, Department of Physics and Neuroscience and Institute for Brain and Neural Systems, Brown University, Providence, RI 02912

In this paper we study the ability of cortical cells to concurrrrently develop orientation selectivity and ocular dominance. We examine whether two proposed learning rules BCM, and a stabilized Hebbian Rule (PACM) can accomplish this aim. In addition we study how initial misalignment affects the binocularity of mature cells. We chose a realistic visual environment, composed of natural scenes that have been preprocessed by center-surround filters. Each neuron is subjected to partially overlapped, partially occluded, of the images originating from both eyes. We examine how changing the degree of the overlap, affects the properties of the mature cells. We find that for both learning rules the receptive fields from the two eyes become matched. For the PCA rule, the receptive fields from both eyes are symmetric, and are completely binocular as long as there is an overlap. For BCM neurons on the other hand the receptive fields from both eyes are non symmetric and mature neurons lose binocularity.

(Heture Address: Sackler Faculty of Exact Sciences, Tel-Aviv University.

608.8 CONTEXTUAL INFLUENCES ON VISUAL DISCRIMINATION IN HUMANS AND ON RF PROPERTIES OF CELLS IN STRIATE CORTEX OF ALERT MONKEYS. M.K. Kapadia, C.D. Gilbert* and G. Westheimer. The Rockefeller University, New York, NY 10021.

The context surrounding a feature influences both the perception of its attributes and the response properties of cells with receptive fields (RFs) containing the feature. We studied the context dependency of the responses of striate cortical cells in awake, behaving primates and compared these findings with psychophysical measurements of line detection in human subjects.

We compared the minimum contrast necessary to detect a light bar presented alone and in conjunction with a second, suprathreshold bar. The second bar lowered the contrast detection threshold of the first bar by 40%. The magnitude of this effect was greatest when the two bars were colinear, and was reduced as the bars were further separated along their axis of orientation, by displacing them from colinearity, and by changing their relative orientation. The response properties of some neurons in the primary visual cortex of the alert monkey showed similar dependencies on stimulus configuration, increasing their response to a bar located within the classical RF when an iso-oriented, colinear bar was presented simultaneously in the RF surround. The second bar, when presented alone, elicited either no response or a suppression of background firing. The response facilitation often declined in bar separation and orientation contrast in a fashion similar to that observed in the psychophysical studies. The plexus of long-range horizontal connections in visual cortex provides a likely substrate of these effects by connecting cells with similar orientation preferences in disparate parts of the visual field.

(Supported by NIH grant EY07968 and a McKnight Development Award)


Previously we have shown that when stimulated by moving contours layer VI cells in the visual cortex provide a feedback influence that causes correlated firing in LGN cell pairs with receptive field alignments appropriate to signalling the presence of the contour. This may serve to increase the synaptic effectiveness of their input to layer IV cells in the cortex. The layer VI cells projecting to the LGN also project to layer IV but as yet we do not know how or whether they influence the synchronisation of layer IV cells directly. With this in mind we have examined the way the spatial extent, orientation and direction of motion of a concentric bipartite grating stimulus influences the response and correlated activity of groups of cells in recorded layer III/IV and VI. The shuffled corrected cross-correlogram revealed highly significant synchronisation compatible with common mode input in cells with common orientation tuning and overlapping fields. This held for responses obtained throughout their orientation tuning curve. We observed that varying the orientation of the outer stimulus could profoundly modify the response to the inner stimulus in the receptive fields of cells with both colinear and monkey optical images. We have also found that connections from orientation columns tuned to quite different orientations to those of the cell in question could influence its response. Under these circumstances we found evidence of connectivity between cells of different orientation for specific stimulus configurations only. We suggest that the input from layer VI cells may contribute to the common mode input correlation of the firing of cells with like orientation selectivity. Coarse and variable cortical circuitry underpins the effects seen under more complex stimulus situations.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
REDUCING BIOPHYSICALLY DETAILED SINGLE CELL MODELS TO SINGLE COMPARTMENT MODELS C. Koch, Ö. Bernander, R. J. Douglas, M.A. Malowald. Caltech, 139-74, Pasadena, CA, 91125, (2) MRC Anatomical Neuropharmacology Unit, Oxford QX1 2QT, UK.

Simulations of biophysically detailed neurons that explicitly model the dendritic tree and incorporate multiple active conductances are computationally too intensive to be feasible in the study of large networks. We therefore derive several highly simplified cell models in a two step approach. First, an intermediate model is derived that is electrically complex (using a Hodgkin-Huxley like formalism) and spatially simple that in the soma and entire dendritic tree are collapsed into a single compartment. This model preserves the response to somatic input, as described by input resistance, f I curves, and voltage and current thresholds. In a second step, the intermediate model is reduced to three yet simpler models: Integrate and Fire (I&F), Diode, and Hopfield units. The I&F model preserves the voltage threshold and generates output spikes whenever the threshold is exceeded. The Diode model is based on the observed exponential relationship between the time-averaged somatic potential (including spikes) and the total membrane current. The Diode and Hopfield models preserve the current threshold and generate a continuous output variable that can be thought of as the mean firing rate. These models represent two extreme viewpoints: pulse or temporal coding assumes that the exact timing of inputs are relevant, while the mean-field viewpoint focuses on the average spike rate.


In brains of higher vertebrates, the functional segregation of local areas that differ in their anatomy and physiology contrasts sharply with their global integration during perception and behavior. We have recently (PNAS 91, 5033) introduced a measure, called neural complexity (Cpq), that captures the interplay between these two fundamental aspects of brain organization. We express functional segregation within a neural system in terms of the relative statistical independence of small subunits of the system and functional integration in terms of significant deviations from independence of large subunits. Cpq is then obtained by estimating the average deviation from statistical independence for subunits of increasing size. Cpq is shown to be high when functional segregation coexists with integration and to be low when the components of a system are either completely independent (segregated) or completely dependent (integrated). We apply this complexity measure in computer simulations of a primary visual area to examine how some basic principles of neuroanatomical organization constrain brain dynamics. We show that the connectivity patterns of the cerebral cortex, such as a high density of connections, strong local connectivity organizing into neuronal groups, patchiness in the connectivity among neuronal groups, and prevalent reciprocal connections, are associated with high values of Cpq. The complexity measure has been used in an initial exploration of data from humans obtained by functional neuroimaging.


Phencyclidine (PCP), ketamine (K) and MK801 are non-competitive NMDA receptor antagonists that decrease injury produced by stroke, trauma and seizures. However, these agents produce vacuoles and kill cortical neurons. We have previously shown that the HSP70 heat shock gene is induced in the pyramidal neurons injured by PCP, K and MK801, that the HSP70 induction is age related, and that anti-psychoctics like haloperidol prevent the vacuoles and HSP70 induction in injured neurons (Sharpe, J. Neurosci Res, 33:605). The present study shows that ketamine also activates microglia around injured and possibly dying neurons in posterior cingulate of adult rats. This effect is dose related, the most microglia being induced at 80-100mg/Kg of ketamine. This effect was also age related, since ketamine did not induce HSP70 or activate microglia in 3, 10 and 20 day old rats, but did induce microglia in progressively older rats. Surprisingly, though haloperidol blocked hsp70 induction by ketamine, haloperidol did not block the activation of microglia in posterior cingulate produced by ketamine. The data is interpreted to mean that haloperidol blocks an injury pathway leading to induction of HSP70, but it does not block the injury pathway leading to microglial activation and possibly neuronal cell death produced by ketamine. The microglial activation and possibly cell death produced by ketamine, PCP and MK801 is not blocked by haloperidol alone, but may require multiple compounds.

A STATISTICAL MEASURE OF COOPERATIVE INTERACTIONS WITHIN CORTICAL NEURONAL GROUPS. J.D. Lamar, G. Tononi* and G.M. Edelman. The Neurosciences Institute, 3377 North Torrey Pines Court, La Jolla, CA 92037.

The neocortex of higher vertebrates is characterized by extensive functional and anatomical compartmentalization at different scales. It has been suggested that, at the scale of a few hundred neurons, the cortex is organized into dynamic and cooperative collections of strongly interconnected neurons, referred to as neuronal groups (G.M. Edelman, Neurosci. 10:115-125, 1993). Here, we investigate a dynamic criterion for the metatile partitioning of a cortical region into neuronal groups, which is based on a statistical measure of the degree of cooperative firing within a local population of neurons. This measure is the normalized third-order moment, or skewness, of the distribution of spike counts cumulated over a local set of simultaneously recorded neurons and a short time interval. The magnitude of positive skewness of the distribution is indicative of the degree of local cooperativity. We show that this skewness is sensitive to the level of coupling among the recorded neurons by means of detailed computer simulations of cortical regions. Such a measure also elucidates local cooperative interactions from firing biases due to shared inputs and, if applied repeatedly over neighboring spatial positions, it can detect statistical borders between neuronal groups. We shall also consider the relationship of a dynamic cortical partitioning of the kind discussed above to underlying factors, such as the activity-dependent changes in the distribution of synaptic strengths and the origin of the organization of axonal termines into segregated cortical patches.


Phencyclidine (PCP), ketamine (K) and MK801 are non-competitive NMDA receptor antagonists that decrease injury produced by stroke, trauma and seizures. However, these agents produce vacuoles and kill cortical neurons. We have previously shown that the HSP70 heat shock gene is induced in the pyramidal neurons injured by PCP, K and MK801, that the HSP70 induction is age related, and that anti-psychoctics like haloperidol prevent the vacuoles and HSP70 induction in injured neurons (Sharpe, J. Neurosci Res, 33:605). The present study shows that ketamine also activates microglia around injured and possibly dying neurons in posterior cingulate of adult rats. This effect is dose related, the most microglia being induced at 80-100mg/Kg of ketamine. This effect was also age related, since ketamine did not induce HSP70 or activate microglia in 3, 10 and 20 day old rats, but did induce microglia in progressively older rats. Surprisingly, though haloperidol blocked hsp70 induction by ketamine, haloperidol did not block the activation of microglia in posterior cingulate produced by ketamine. The data is interpreted to mean that haloperidol blocks an injury pathway leading to induction of HSP70, but it does not block the injury pathway leading to microglial activation and possibly neuronal cell death produced by ketamine. The microglial activation and possibly cell death produced by ketamine, PCP and MK801 is not blocked by haloperidol alone, but may require multiple compounds.


This study examined infiltration of hematogenous cells after occlusion of the middle cerebral artery in rats. Cryostat sections of the infarcts were stained immunocytochemically for CD4, a pan-T cell marker, lymphocyte subsets CD4 and CD8, and ED1, a marker for macrophages. Besides granulocytes, numerous CD5+ T cells were rare, while CD8+ cytotoxic/suppressor lymphocytes were abundant. Moreover, CD8+ lymphocytes greatly outnumbered CD5+ T cells indicating the presence of CD8+/CD5+ natural killer lymphocytes. ED1+ macrophages appeared on day 1 and were still present on day 30. As an early event ICAM-1 was upregulated on cerebral vessels. This study shows that ischemic lesions can lead to lymphocytic infiltration into the CNS. Blocking of lymphocyte-derived cytokines will help to elucidate the functional contribution of immune cells to stroke.
ISCHEMIA:

Xu, Supported FBP spectroscopy L.: H., University FRUCTOSE-1,6-BIPHOSPHATE brain, Nagafuji, 1.73 served middle levels studies). which decreased in vivo Chana, RAT and pre-treatment, there might the hypothesis that neuronal ischemic brain damage has been to be elucidated, our previous results conducted the conclusion that NO might mediate the ischemic cerebral damage. The present study aimed at not measuring neuronal expression in the ischemic brain, employing middle cerebral artery occlusion (MCAo) and reperfusion in rats. The newly developed microsensor was calibrated in NO containing solution, and the microsensor (pH) in oxygenometer (mmHg) and laser CBF flowmetry (ml/100g/min) were implanted in the L. MCA territory. A total of 20 animals were assigned into 4 hour MCAo (saline-N=5), LNA (l-Arg-tryptophan)-treated (N=3) or 2hour MCAo followed by 2hour recontruction (saline-N=5), LNA-treated (N=5). In this setting, the value obtained is not absolute NO concentration but relative to the baseline. The first peak (P1) of NO production, L73 ± 0.19μM, was found at the period of initial 20-30min MCAo. Three and a half hours later another peak was observed (P2, 553 ± 08μM). Also, P3 (0.93 ± 0.2μM) was induced by reperfusion. LNA inhibited or abolished P1-3. The present results support the hypothesis that overproduction of nitric oxide in the brain subjected to permanent and temporary ischemia might lead to the cytotoxic effects of nitric oxide in the ischemic brain.

FRUCTOSE-1,6-BIPHOSPHATE PROTECTS ATP LEVELS IN HYPOXIC NEONATAL RAT BRAIN SLICES. L. Lit*, M.T. Esparol, J. MacDonald, L. Chang, G.A. Gregory, P. R. Weinstein, T. James, and P-H. Chan.

University of California, San Francisco, California 94143

In vivo administration of fructose-1,6-biphosphatase (FBP) provides hemodynamic and metabolic protection during ischemia and hypoxia.2 However, mechanism of protection have not yet been elucidated, and, in turn, appear to not work in certain circumstances. We applied ex vivo NMR spectroscopy techniques, recently developed for studies of live rat brain tissue, to examine if there is direct metabolic protection by FBP in hypoxic (PO2<0 mm Hg) neonatal (7-14 days old Sprague Dawley) slices (350). Total acquisition time for interleaved 31P/H spectra was 5 minutes. With no pre-treatment, metabolic impairment was seen in 31P spectra obtained at 5 min of hypoxia, at which time the Lactate/NAA ratio was increased by a factor of 3. Pre-treatment with FBP decreased ATP loss and lactate production, and increased the rate of recovery during reoxygenation. Post-treatment with FBP did not protect the nearly full recovery found with pre-treatment. In this study protection comes primarily from intracellular effects in the tissue of interest, and systemic response such as calcium protection and increased blood flow. Mechanisms of FBP's protection are not understood and additional studies are needed. Hypotheses for FBP protection point to the involvement of calcium homeostasis, reduction of free-radical injury, and increased pentose phosphate pathway. Supported by NIH Grants GM54376, NS 2202, NS15435, NS25372, and 1RO1 HD 3941, Research (1) Keleher JA, Gregory GA, and Chan P-H. Neurochem. Methods 19:209-215, 1994. (2) Esparol MT, Litt L, Xu Y et al. J Cereb Blood Flow Metab. 14:269-278, 1994.
609.9
INDUCTION OF PROTECTIVE MECHANISMS IN CEREBRAL ISCHEMIA 1 N. Kawahara, 2 C.A. Reutzel, 2 B.W. Wiegand, 2 S. Costioli and 1 L. Klatzow*. 1Stroke Branch, NINDS, NIH, Bethesda, MD 20892 and 2Regeneron Pharmaceuticals Inc., Tarrytown, NY 10591.

Our study is based on serendipitous finding indicating brain ischemia induced by injection of Sprague-Dawley rats, by local application of KCl. Assays on brain tissue after SD revealed that: 1. Increased c-Fos expression in the neurons of the cerebral cortex on the side of KCl application and bilaterally in the diencephal gyri; 2) No enhanced HSP-70 expression, 3) Conspicuous increase in protein synthesis measured by 3H-leucine incorporation associated with a pronounced reduction in deoxyglucose utilization, both demonstrable in ipsilateral hemisphere 3 days after SD and 4) Upregulation of mRNA for the brain-derived neurotrophic factor (BDNF), conspicuously noticeable at 4 h and 3 days after SD.

Our studies indicate that a subethal stress, such as SD, is capable of inducing a temporary state of resistance to cerebral ischemia, in which stimulation of protein synthesis and neurotrophic factors may play a significant role.

609.11
CHARACTERIZATION OF INDUCED ISCHEMIC TOLERANCE IN FOCAL CEREBRAL ISCHEMIA IN RATS. 1 R.P. Simon, 2 K. Chen, 2 R.A. Swanson* and 3 S.H. Graham. Neurology, University of California, San Francisco, CA 94143.

Background: Brief periods of non-lethal global ischemia have been reported to induce resistance against subsequent lethal ischemic injury in hippocampal neurons. We determined if a similar phenomenon occurs after focal ischemia.

Methods: Ninety-four adult Sprague-Dawley rats underwent either sham operation or brief intervals of focal ischemia by using the MCA/MCAo system. Twenty-four hours later the MCAa were isolated and the rats were subjected to 45 min ischemia. HSP70, HSP60 and HSP27 protein expression were assessed by immunoblotting. Results: Upon reperfusion the levels of these HSPs increased significantly compared to sham-operated rats.

Discussion: The induction of HSP expression during initial ischemia may play a role in protecting brain tissue during subsequent ischemic episodes.

609.12

We have demonstrated that chronic electrical stimulation of the FN of the adult rat increases cerebral blood flow (CBF) and reduces ischemic damage when cerebral blood flow is reduced by induced unilateral occlusion of the middle cerebral artery. This neuroprotection is not observed in young rats. In order to understand the nature of this protection, we have studied the effects of FN stimulation on the factors that are known to influence CBF.

The results of these studies suggest that the protection observed in the adult rat is due to an increase in the production of HSPs, which play a role in protecting brain tissue during ischemic episodes.

NEUROTROPIC FACTORS: EXPRESSION AND REGULATION VII

610.1
NGF-HIGH-EXPRESSING TRANSGENIC MICE CONTAIN INCREASED NUMBER OF PGP 9.5-IMMUNOREACTIVE FIBERS IN THE VOMERINOASAL ORGAN. 1 S. Takam*1, M.J. Getchell 2, 2 K. Albers* 2, M. Yamagishi* 2, T.Y. Getchell 2, 3, 4. Dept of Physiol., 2Sanders-Brown Ctr. on Aging, 3Div. of Otolaryngology, 4Dept of Pathology, University of Kentucky College of Medicine, Lexington, KY 40536.

The expression of protein gene product (PGP) 9.5 in extrinsic nerve fibers of the vomeronasal organ (VNO)-neighboring respiratory epithelium (RE), and the associated glands of the nasal cavity, is regulated by the expression of the NGF nerve growth factor (NGF)-high-expressing 6-week-old transgenic mice that contain the gene isolated from the 14-fold NGF-expressed rats. Increased density of extrinsic PGP 9.5-immunoreactive (IR) fibers was observed in the VNO-RE, and the glands of the transgenic mice. The soft palate, whiter pad tongue and nasal mucosa of the mice, which were used as positive controls, all demonstrated a marked increase in the density of extrinsic PGP 9.5-IR nerve fibers. The expression of transgenic PGP 9.5-IR nerve fibers were significantly greater in the VNO-RE (12.8 X control, P<0.01) and RE (3.5 X control, P<0.01) of the transgenic mice. Similar expression was observed in the VNO-RE and RE, these cells in the transgenic mice presumably contain the NGF transgene, resulting in continuous production and secretion of NGF, which induced significant hyperinnervation of the VNO-RE and RE. This hypothesis is supported by the fact that basal cells in the epithelia of positive tissue controls of transgenic mice express both K14 and NGF.

Supported by NIH grants DC-01595 (TVG) and DC-01715 (MLG).

610.2
MECHANICAL INJURY INCREASES bFGF AND CNF EXPRESSION IN THE RAT RETINA. 1 Rong Wen, 2 Ying Song, 3 Michael T. Mathes, 1 Douglas Yauamura, 2 George D. Yancopoulos, 4 Matthew L. LaVail, 5 and Ron J. Steinberg*. 1Dept. of Physiology, Ophthamology, and Anatomy, UCSF, San Francisco, CA 94143, and 2Regeneron Pharmaceuticals Inc., Tarrytown, NY 10591.

It has been shown that mechanical injury to the retina prevents photoreceptors from degenerating in the RCS rat with an inherited retinal degeneration, and in the light afferent field, which indicates that mechanical injury activates intrinsic retinal mechanisms that promote photoreceptor survival. To find out if factors that show neurotrophic activity are involved in injury-induced photoreceptor survival, we assessed the expression of bFGF, CNF, BDNF, and IGF-1 following mechanical injury. The retina was injured by making an incision through the choroidal blood vessel, that penetrated the subretinal space of each eye of an adult Sprague-Dawley rat. Control animals were without injury. Retinas were taken 0.5, 1, 2, 4, 7, or 10 days post-injury. Northern blot analysis showed marked increased mRNA of bFGF following injury. Compared to controls, expression of bFGF increased by more than 6-fold at 12 h post-injury; peaked at 2 days post-injury (more than 7-fold); and was still at a 5-fold level at 10 days post-injury. Expression of CNF increased more than 2-fold at 1 day post-injury; peaked at 4 days post-injury (about 5-fold); and was still at a 2-fold elevation 10 days post-injury. In situ hybridization showed that both bFGF and CNF were expressed in the inner nuclear layer and were more concentrated around the outer boundary. BDNF and IGF-1 did not show significant changes in expression levels of both bFGF and CNF up-regulate in the retina following mechanical injury, with the bFGF effect being earlier and greater than that of CNF. We conclude that these two factors are likely to be involved in injury-induced photoreceptor rescue.

Supported by a Grant-in-Aid from the Fight for Sight Research Division, National Society to Prevent Blindness, Inc.; R41RC40173, USCF to RW; by NIH grant EY01919 to MML; by NIH grant EY01429; and funds from the Reinitis Pigmentosa Foundation to RHS.
610.3

CDF/LIF mRNA INCREASES FOLLOWING CORTICAL INJURY. N. N. Mouyei, L. R. Banner* and P. H. Patterson. Biology Division, California Institute of Technology, Pasadena, CA 91125.

The neurotrophic cytokine cholinergic differentiation factor/leukemia inhibitory factor (CDF/LIF), can act both as a trophic factor, enhancing neuronal survival, and as a differentiation factor to alter neuronal gene expression. CDF/LIF also plays a role in the response of adult neural tissue to injury. When peripheral nerves are transected, CDF/LIF mRNA is dramatically up-regulated near the site of injury (Banner & Patterson). Moreover, the neuronal response to nerve section is much reduced in LIF knockout mice (Rao et al., Neuron 11:1175, 93).

To test whether CDF/LIF expression is regulated in a similar manner after injury in the CNS, surgical lesions were made in adult rat cortex. Using a quantitative RNase protection assay, we find that CDF/LIF mRNA increases significantly relative to the contralateral cortex and to anesthesia-treated controls. The increase in CDF/LIF expression begins within 6 h after injury and reaches a peak at 24 h. CDF/LIF mRNA expression returns to baseline values by 7 days post-injury. Thus, this cytokine is likely to play a role in the response to adult brain injury as it dose in the periphery.

610.5


It has been shown before that several species that peripheral glial cells express receptors for nerve growth factor during development as well as following nerve injury. The functional significance of these glial receptors in the biology of NGF is not well understood. Since embryonic glial cells as well as glial cells in lesioned peripheral nerves also express NGF mRNA, we have explored whether NGF would affect NGF (neurotrophin) secretion by these cells.

Glial cells purified by a rapid procedure from chicken embryonic sensory ganglia were cultured in the presence of various concentrations of NGF. After two days conditioned media (CM) were harvested and assayed for the presence of neurotrophic activity in a single neuron bioassay. Anti-NGF antibodies were employed to probe for NGF in the CM.

Glial cells cultured without NGF were found to secrete neurite growth promoting activity which was not inhibited by antibodies against NGF. However, if the glial cultures were spinkled with very low concentrations of NGF (≤2pM), NGF-like activity in the medium rose dramatically (up to 50 fold). This was not observed, if NGF had been added to the glial cultures at 50 times higher concentrations.

It is concluded that the availability of neurotrophins during development may not only be regulated by the target of innervation but also by glial cells which appear to have the capacity to sense lines of NGF and respond in an autocrine fashion, finely tuned to the NGF concentrations present extracellularly. An autocrine function of glial NGF receptors can be expected to be of critical importance for morphogenetic effects of nerve development and in nerve regeneration. Supported by DFG ZI 1963/4 and ZI 455/2-2.

610.6

NERVE GROWTH FACTOR REGULATES THE EXPRESSION AND KINASE ACTIVITY OF p32cdc2 AND p34cdc2. K. J. Buchkovitch* and E. Ziff. New York University Medical Center and Howard Hughes Medical Institute, 550 First Avenue, New York, NY 10016.

Nerve growth factor (NGF) promotes the survival and differentiation of the pheochromocytoma cell line PC12. The function of NGF as both a survival and differentiation factor may be related to its ability to regulate PC12 cell cycle progression. NGF treatment of PC12 cells leads to a decrease in the percentage of cells in the DNA synthesis phase (S phase) of the cell cycle and an accompanying increase in G2 phase cells. We have investigated the mechanism by which NGF regulates PC12 cell cycling. Specifically, we have analyzed the regulation of two cyclin-dependent kinases, p33cdc2 and p34cdc2, which are required for progression through the G1/S and G2/M cell cycle transitions of the cell cycle. NGF treatment lead to a decrease in the steady state levels of p32cdc2 and p34cdc2, p32cdc2 and p34cdc2 form complexes with regulatory molecules known as cyclins. NGF treatments resulted in decreases in the kinase activity of several cyclins p32cdc2 and cyclin p34cdc2 complexes. The decreases in kinase activity could be attributed in part to the decreases in the steady state levels of p32cdc2 and p34cdc2. The timing of the down regulation of the kinases was dependent on the level of p140Raf NGF receptor in the cells. A clonal cell line that overexpressed p140Raf and differentiated with accelerated kinetics (BL Hempstead et al, 1992) also down-regulated the kinases with accelerated kinetics. This suggested that the rate of the decrease in p32cdc2 and p34cdc2 kinase activity was dependent on the level of p140Raf receptors and the strength of the NGF signal.

610.8


S-100 protein is a protein that is expressed in the neuron and glial cells of the nervous system. Previous studies have shown that it is synthesized in rat brain in response to NGF treatment, and that it is not synthesized in response to BDNF treatment. However, the current study shows that S-100 protein is synthesized in response to NGF treatment in human brain tissue. In fact, the current study shows that the levels of S-100 protein are increased in response to NGF treatment in human brain tissue. The current study also shows that the levels of S-100 protein are increased in response to NGF treatment in human brain tissue.

We report that both NGF-2 and IL-18 regulate the S-100 gene in astrocytes from the cerebral cortex, hippocampus, and hypothalamus. Total RNA was isolated from cultures, purified, and subjected to the solution hybridization assay using highly specific antagonists for rat S-100 and rat cyclinB, an internal standard which is not regulated by either NGF-2 or IL-18. We find that S-100 mRNA levels decrease 2- to 3-fold relative to controls in response to 24 or 48 hours of treatment with either 10 ng/ml NGF-2 or 10 U/ml IL-18 alone, and decrease 5- to 10-fold in response to combination treatment at the same doses over the longer term. Interestingly, in the presence of NGF-2, increases S-100 gene expression two-fold over control levels in cerebral cortical astrocytes. Furthermore, a similar time-course and at the same doses, we find that only NGF-2 suppressed S-100 mRNA levels in 24 and 48 h of treatment with NGF-2 has no effect on gene expression. These data demonstrate that both NGF-2 and IL-18 regulate astrocyte S-100 mRNA levels, which may be an important mechanism through which these factors modulate neuron-glia interactions.
61.0.9 CHARACTERIZATION OF NEUROTROPHIN-3 IMMUNEACTIVE SENSORY NEURONS IN RAT DORSAL ROOT GANGLIA

X.F. Zhou, C. Chen and R.A. Rush, Department of Human Physiology, University of Nebraska, Lincoln.

Neurotrophin-3 (NT-3) is a member of the NGF family known to support survival of sensory neurons in vitro but its physiological roles are not known. We recently reported, using a newly generated antibody, that a subpopulation of rat sensory neurons contained NT-3 like immunoreactivity (R. Zhou & Rush, 1993, Brain Res. 621, 189-199). In the present paper, we have identified NT-3-like immunoreactivity to be localized primarily in large sensory neurons. These NT-3-positive neurons were detected in cervical (56% of total) and lumbar (38%) but not in thoracic dorsal root ganglia (DRG; 18%).

61.0.10 REGULATION OF NEUROTROPHIN-3 BY BRAIN DERIVED NEUROTROPHIC FACTOR IN HYPOTHALAMIC MOTOR NEURONS.

R.A. Rush*, X.F. Zhou and D. Greenson, Department of Physiology, University of Arizona, Tucson, AZ 85724.

Identification of the cellular location of the neurotrophins has so far proved difficult, primarily due to the long half-lives and poor tissue specificity for techniques for neurotrophin immunohistochemistry. Recently we have generated antisera to peptide sequences of neurotrophin-3 (NT-3) which have proved useful for immunohistochemistry (Zhou and Rush, Brain Research, 621:188-199,1993). Purified antibodies from this serum show no cross-reactivity with nerve growth factor (NGF) or brain derived neurotrophic factor (BDNF). Within the brain the only fiber that is confined to motor neurons, consistent with in situ hybridization studies for mRNANT-3. We now show that unilateral lesion of the hypogasalus nerve leads to the loss of NT-3-immunoreactivity (IR) from the ipsilateral hypogasal nuclear mass. This is accomplished by a reduction of cholergic agent/transporter-IR (CHAT), but an up-regulation of both low affinity nerve growth factor receptor (LNGFR) and choline acetyltransferase activity. However, application of BDNF to the lesioned nerve leads to restoration of the NT-3-IR and Chat-IR, but does not reverse the up-regulated LNGFR or cholinergic activity. Our findings support the view that BDNF can regulate the synthesis of NT3 via a specific intracellular pathway, thereby providing a mechanism by which neuronal connectivity can be controlled.

61.1.1 HUMAN DELTA OPIOID RECEPTOR: CLONING, EXPRESSION AND PHARMACOLOGICAL CHARACTERIZATION.


University of Arizona Dep. of Pharmacology, Tucson, AZ 85724.

Antisense oligos selective for δ opioid receptor have fewer side effects than ligands selectively acting at μ or κ opioid receptors. Recently, we cloned a human δ opioid receptor for exclusive characterization in ligand requirements. The cloning was based on hybridization screening of several human cDNA libraries. The probe was obtained by FCR from the mouse δ opioid receptor cDNA and hybridized to a clone in a human stomach cDNA library that lacked the 5' end of the ORF. The probe obtained from this clone was used to identify the clone from human temporal cortex cDNA library that contained the missing upstream part of the ORF but ended before the termination codon. These two clones were ligated together using HindIII in E.coli present in the overlapping cDNA region. It is now shown that the ligated clones encode for the same receptor using southern blot analysis of human genomic DNA. Two probes, one present in δ opioid receptor cDNA clone and second obtained from 3' end of human striatum cDNA clone, gave a band of the same size (about 4.6 kb) while hybridizing to human genomic DNA digested with BamHI. The receptor is localized in the human temporal cortex and hippocampus. These observations indicate that the human δ opioid receptor is a homologous sequence.

61.1.2 6-OPIOD RECEPTOR IMMUNEACTIVITY: RELATIONSHIP TO BIOMIC ANENMMES AND ENKEPHALIN IN RAT SPINAL CORD AND BRAIN STEM.


Department of Anatomy and Neurobiology, University of Pennsylvania, Philadelphia, PA 19104.

During the development of neurotransmitter systems of the central nervous system, there is a close correlation between the localization of receptors, transporters and the synthesis of neurotransmitters. Utilizing an antisense oligo to synthesize a delta opioid receptor (OPRD), we have been able to generate antibodies specific to the receptor. In the present study, we have used OPRD antisense oligos to probe for the presence of OPRD mRNA in the rodent nervous system. The results of these studies, demonstrate a close correlation between the presence of OPRD mRNA and the presence of a specific opioid receptor. These results suggest that the delta opioid receptor is a potential target for the modulation of opioid receptor function in the nervous system.

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611.3 CELLULAR LOCALIZATION OF A \( \mu \) OPIOID RECEPTOR (MOR) IN RAT BRAIN AND SPINAL CORD. 1. A. Arvidson1*, M. Redl1, J. H. Lee1, A. Nakada2, D. J. Dove2, S. Chakrabarti2, H. Li1, B. P. Lass2, L. Y. Wu, M. W. Wessendorf1 and R. Eide1. 1Dept. Cell Biology and Neuroanatomy and 2Dept. Pharmacology, University of Minnesota, Minneapolis, MN 55455; 3Dept. Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN 46222.

The \( \mu \) opioid receptor (MOR) was localized by immunohistochemistry using antisera raised against a synthetic peptide corresponding to the C-terminal of the predicted amino acid sequence for the \( \mu \) opioid receptor. The MOR antisera immunoprecipitated I-125 \( \beta \)-endorphin cross-linked membrane proteins with mw's of 55-69 kDa that had been isolated from a cell line stably expressing \( \mu \) opioid receptor cDNA. Protein of similar mw were labeled by MOR antisera using Western blots. In addition, the MOR antisera stained cells transfected with native and epitope-tagged \( \mu \) opioid receptors. MOR-like immunoreactivity was frequently observed in neuronal membranes, both in the somatic and dendritic domains. However, in some areas such as in the dorsal horn, axon labeling could also be demonstrated. In the brain MOR-like immunoreactivity showed an excellent correlation with the distribution of mRNA encoding MOR, and also with earlier \( \mu \) opioid receptor binding studies. The distribution of enkephalin, the putative endogenous ligand for this receptor, showed a distribution complementary to that of MOR in many areas in the brain and spinal cord. However, double-labeling was not observed. These findings suggest that the CNS \( \mu \) MOR receptors are expressed in somatic and dendritic membranes, however, neurons also exist that transport MOR to the axon terminal. Supported by NIDA.

611.5 CELLULAR DISTRIBUTION OF \( \mu \), \( \delta \) AND \( \kappa \) OPIOID RECEPTOR mRNA'S IN HUMAN CNS. B. Anton1*, K. Mori1, M. Huh1, D. K. Kaufman1, E. Stickle2, T. Mauro1, T. Tran1, and C. J. Evans, Dept. of Pharmacology and the Neurology Branch of the National Institute of Neurological Disorders and Stroke, Los Angeles Ca 90024, and Dept. of Pathology, Veterans Administration Hospital, Little Rock, Arkansas.

The cloning and sequence analysis of cDNAs encoding \( \mu \), \( \delta \) and \( \kappa \) opioid receptor classes have allowed generation of specific nucleic acid probes to be used in experiments to correlate mRNA expression to the anatomical localization of cells expressing these opioid receptor classes in both mouse and rat brain. We have recently isolated genomic clones encoding the human \( \mu \), \( \delta \) and \( \kappa \) opioid receptors. Sequence information derived from these clones allowed us to generate specific cDNA probes to examine the expression patterns of these receptors within the human brain. We have also been able to detect the expression of \( \mu \), \( \delta \) and \( \kappa \) opioid receptor mRNAs in human tissue. Cerebrospinal fluid containing the three opioid receptor transcripts were found in the major human cerebral folia allowing distinct laminar and expression patterns within the 6 cortical layers. Cells containing the \( \mu \), \( \delta \) and \( \kappa \) mRNA sequence specific distribution were detected whereas cells containing \( \kappa \) mRNA seem to be localized predominantly in clusters, suggesting a possible patch-like distribution for this latter opioid receptor subtype in human caudate-putamen. Co-labeling experiments coupling opioid receptor in situ hybridization to choline acetyltransferase (ChAT) immunochemistry to explore preferential patch-matrix distribution of opioid receptors in human caudate-putamen are currently in progress as is the anatomical mapping of other human brain regions. Supported by a NIDA grant #DA05010 and the W.M. Keck Foundation.

611.6 KAPPA-OPIOID RECEPTOR AGONISTS SUPPRESS VOLTAGE-ACTIVATED POTASSIUM CURRENT IN CATHA CELLS. S.C. Baraban, E.W. Deitch, P.G. Gigante, and E. Friedman, Dept. of Pharmacology and Neurology, University of Virginia, Charlottesville, VA 22908.

The CATHa cell line is derived from tyrosine hydroxylase (TH)-positive tumors in transgenic mice. CATHa cells have a high density of \( \delta \) and \( \kappa \) opioid receptors (Suri et al, J. Neurosci. 13:1280, 1993). In this study we examined the opioid sensitivity of CATHa cells using whole-cell voltage-clamp techniques. Depolarizing commands to mid-membrane potential (holding potential +60 mV) produced sustained outward current (I_o). External tetraethylammonium (25 mM) reduced I_o by 40-60% (n=10). Depolarizing steps following a hyperpolarizing pre-pulse (-110 mV) elicited fast, transient outward current (I_t). I_t was selectively blocked by 3-aminopropionic acid (10 mM; n=5). Intracellular dialysis with TEA (25 mM) and CaCl_2 (150 mM) blocked voltage-activated potassium currents (n=5).

Phenyl (0.5-100 \( \mu \)M) reduced I_o, in a concentration dependent manner (EC_50 = 3.5 \( \mu \)M, max. inh. = 55%, n=30). Naloxone (10 \( \mu \)M) blocked the inhibitory effect of 10 \( \mu \)M morphine (n=8). The \( \mu \)-specific agonist DAGO (2.5 \( \mu \)M; n=6) and the \( \kappa \)-specific agonist DPDPE (2.5 \( \mu \)M; n=7) produced no effect. USO,488 (0.3-50 \( \mu \)M) and specific, reduced I_o in a concentration-dependent manner (EC_50 = 3.5 \( \mu \)M, max. inh. = 46%, n=8). The \( \kappa \)-specific agonist receptor antagonist RU-5,698 (10 \( \mu \)M) blocked the inhibitory effect of 10 \( \mu \)M morphine (n=5) or 10 \( \mu \)M USO,488 (n=7). Kappa-receptor mediated current reduction was prevented by intracellular dialysis with an inactive form of GDP (GDP-\( \beta \)-5 100-200 \( \mu \)M, 3.5-5 min, n=10). Incubation with perisotia tola (PTX; 500 ng/ml; 24 hr; n=10) did not couple kappa-opioid induced suppression of I_o. These results suggest that opioid suppression of I_o in CATHa cells is mediated by a kappa opioid receptor coupled to a PTX-insensitive G-protein.
**611.9**

Opiate Receptor Structure/Function Relationships and Molecular Modeling

Christopher K. Surratt*; Peter S. Johnson**; Akatsuki Morishita; Brian K. Sedlack; Carrie J. Blanchard; Jiu-Ben Wang; Luca C. Vahabzadeh and George R. Uhde*  

*Molecular Neurobiology Branch, Office of the Director, Intramural Research Program/NINDA, National Institutes of Health; **Scripps Research Institute, La Jolla, CA, USA

Opioid receptor cDNAs encode poorly conserved N- and C-terminal regions and conserved, charged transmembrane domain residues. Deletion of 64 N-terminal amino acids yields a receptor that binds DAMGO and reduces the affinity of DAMGO and other agonists. The complete receptor was expressed in Chinese hamster ovary cells and was purified by affinity chromatography. The purified receptor was tested in a radioligand binding assay and was shown to have high affinity for DAMGO and other opioids. The results suggest that the N- and C-terminal regions of the opioid receptor play a role in the binding of ligands and in the regulation of receptor activity.

**611.10**

CONSTRUCTION, EXPRESSION AND CHARACTERIZATION OF EPITOPE-TAGGED OPIOID RECEPTORS R.L. Zastawny Sr., S.R. George, R. Brown; Uribena; and B.F. O'Dowd  

Department of Pharmacology, University of Toronto, Toronto, Ontario, CANADA M5S 1A8

We have previously reported the cloning, pharmacological characterization and synthesis of a new opioid receptor antagonist, ET-743, which was found to be a potent and selective antagonist of the mu, kappa and delta opioid receptors. In the present study, we have used the cDNA expression system to express the ET-743 epitope-tagged receptor in COS-7 cells. The receptor was expressed and purified as a GST-fusion protein and was shown to have the same pharmacological properties as the wild-type receptor. The results provide a valuable tool for the study of opioid receptor function and the development of new therapeutic agents.

**611.11**

MORPHOLOGIC ALTERATIONS IN THE HYPOTHALAMUS OF RATS WITH OPIOID THERAPY FOR CHRONIC PAIN R. M. Rasmussen, H. Hjorth, L. B. Christiansen, and S. L. George  

Department of Pharmacology, University of Toronto, Toronto, ON, Canada

We have examined the effects of chronic opioid therapy on the morphology of the hypothalamus in rats. The results indicate that chronic opioid therapy leads to changes in the size and structure of the hypothalamic nuclei, as well as to alterations in the expression of neuropeptides and other markers of neuronal activity. These changes suggest that chronic opioid therapy may have long-term effects on the function of the hypothalamus and on the regulation of feeding and other homeostatic processes.

**611.12**


The Johns Hopkins Medical Institutions, Baltimore, MD

We have quantified the deactivation of the human brain in response to opioid administration using PET and [11C] METHYL NALTREXONE. Our results demonstrate that the deactivation of the primary motor cortex is greater in response to opioid administration than to placebo, suggesting a role for opioid receptors in the modulation of motor function.

**612.1**

COMPARISON OF A BRAIN PURIFIED METALLOPROTEASE WITH HUMAN CATHEPSINS G AND D: EVALUATION OF SEQUENCE SPECIFICITY USING PEPTIDE SUBSTRATES AND REGIONAL SELECTIVITY USING PURIFIED BRAIN APP I. S. Rosenberg-Reits*; A. S. Brown, D. M. Tumolo, M. A. Snyurt, and J. E. Jacobson  

Department of Central Nervous System Biological Research, Lederle Laboratories, Division of American Cyanamid, Pearl River, NY. 10965

A number of proteases have been proposed as candidates for the activity that cleaves at the amine-terminus of β-amyloid peptide (β-AP). No single protease has emerged as possessing all the required properties for β-secretase including sequence specificity, ability to cleave amyloid precursor protein (APP) appropriately, brain localization and correct sensitivity to sequence substitution. We have recently purified another candidate β-secretase, a 35-50 kDa metalloproteinase purified from monkey brain and found its activity using a synthetic peptide substrate (SEVMDKMDAEP) which flanks the N-terminal of βAP. The assay and amino acid analysis reveal that the major cleavage is between Lys and Arg. The enzyme is inhibited by EDTA, but not by EDTA. The pH optimum for this protease is 5.0-6.0, but activity is still retained at physiological pH.

We have compared three candidate proteases (catalyst D, cathepsin G, and the above metallopeptase) for their ability to cleave, in addition to SEVMDKMDAEP, a synthetic peptide substrate, dipalmitoylated Lys-Mp (1-Anilino-8-naphthalene) (Lys-Mp) which is a substrate for cathepsin G. The enzyme is inhibited by EDTA, but not by DTT or PMSF. The pH optimum for this protease is 5.0-6.0, but activity is still retained at physiological pH.

**612.2**

INCREASED NEUROPLASTIC CATHEPSIN D GENE EXPRESSION AND ACTIVE RELEASE OF COMPETENT PROTEASE IN ALZHEIMER DISEASE R.A. Nixon*, A. M. Casado, A. L. Scherzer, N. Kovass, L. A. Kasac-Sanderson, McLean Hospital, Harvard Medical School, Belmont, MA

We have found that activation of the tyrosinase system, evidenced by increased cathepsin gene expression and accumulation of secretory and tertiary lysosomes, is an early marker of synaptic dysfunction in Alzheimer disease (AD). We have demonstrated that cathepsin D, a 90-kDa form of the enzyme, accumulates in lysosomes of neurons in post-mortem AD brains. In addition, we have found that the expression of cathepsin D mRNA is increased in the hippocampus of AD brains compared to control brains. Our results suggest that the accumulation of cathepsin D in AD brains is mediated through a mechanism that involves the activation of the tyrosinase system.

**DEGENERATIVE DISEASE: ALZHEIMER’S—BETA AMYLOID X**

**612.1**

COMPARISON OF A BRAIN PURIFIED METALLOPROTEASE WITH HUMAN CATHEPSINS G AND D: EVALUATION OF SEQUENCE SPECIFICITY USING PEPTIDE SUBSTRATES AND REGIONAL SELECTIVITY USING PURIFIED BRAIN APP I. S. Rosenberg-Reits*; A. S. Brown, D. M. Tumolo, M. A. Snyurt, and J. E. Jacobson  

Department of Central Nervous System Biological Research, Lederle Laboratories, Division of American Cyanamid, Pearl River, NY. 10965

A number of proteases have been proposed as candidates for the activity that cleaves at the amine-terminus of β-amyloid peptide (β-AP). No single protease has emerged as possessing all the required properties for β-secretase including sequence specificity, ability to cleave amyloid precursor protein (APP) appropriately, brain localization and correct sensitivity to sequence substitution. We have recently purified another candidate β-secretase, a 35-50 kDa metalloproteinase purified from monkey brain and found its activity using a synthetic peptide substrate (SEVMDKMDAEP) which flanks the N-terminal of βAP. The assay and amino acid analysis reveal that the major cleavage is between Lys and Arg. The enzyme is inhibited by EDTA, but not by EDTA. The pH optimum for this protease is 5.0-6.0, but activity is still retained at physiological pH.

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**612.2**

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Alzheimer's disease (AD) is characterized by the deposition of β-amyloid protein (Aβ) in brain parenchyma and blood vessels. The formation of Aβ requires its proteolytic release from a larger molecule, the amyloid precursor protein (APP). Recently, we have purified and characterized a protease from human brain which is a candidate for an amyloidogenic APP processing enzyme. The enzyme is highly homologous to the rat metallo-
endopeptidase (MMP-9). It cleaves a carboxyl peptide ranking the N-terminus of Aβ at the Met-Asp bond and is capable of generating a 15 kDa amyloidogenic fragment from recombinant human APP in vivo.

To characterize the enzyme further, we developed monoclonal anti-
body against a 20 amino acid peptide derived from the sequence of the purified protease. These antibodies were used to characterize the tissue distribution and the cellular localization of the enzyme, using immuno-
histochemistry, immunoprecipitation and Western blots. The enzyme is present in all monkey tissues examined, with highest amounts being found in brain, testis and lung, as well as a variety of other tissues. In human brain, astrocytes were stained strongly. In human cultured cells, the metalloprotease was found in neuroblastoma and kidney cells but not in glioblastoma cells. Upon a rabbit retina/optic nerve system in vivo, we purified by equilib-
rium sucrose density gradient centrifugation three fractions containing transport vesicles. The optic nerves of APP and ApoE in the same vesicular compartments.

612.5 INTRACELLULAR AND EXTRACELLULAR ACCUMULATION OF AMYLOID Aβ PEPTIDE BY DIFFERENTIATED PC12 CELLS. Debra Burdick* and Charles G. Gilbe, Department of Molecular, Biol. and Biochem. Univ. of Calif., Irvine, CA 92717.
The mechanisms of amyloid accumulation in Alzheimer’s disease are largely unknown, but may be central to the etiology of the disease. Our previous results have demonstrated that Aβ1-42 accumulates in late endosomes, or secondary lysosomes of human fibroblasts and is resistant to degradation. We have extended these investigations to NFG-differentiated PC12 cells, and we find that while Aβ1-42 also accumulates in these cells, there are a number of differences from the results obtained with fibroblasts. Immunofluorescent confocal microscopy indicates a dual localization of the accumulated Aβ1-42, intracellular, punctate granules and aggregates of peptide at the cell surface. Unlike fibroblasts, the Aβ1-42 on the surface of PC12 cells is largely resistant to removal by trypsin. In contrast to fibroblasts, the shorter amyloidogenic peptides, Aβ1-26 and Aβ1-40, accumulate in PC12 cells but to a lesser degree than Aβ1-42. The accumulated Aβ1-42 is stable for at least 3 days while Aβ1-28 and Aβ1-40 are degraded within 90 min after exchanging the culture medium with fresh medium. The intracellular Aβ1-42 is located in a dense organelar compartment which overlaps the distribution of late endosomes and lysosomes and colocalizes with internalized horseradish peroxidase which has been targeted to lysosomes. The internalized Aβ1-42 is not sequenceable and highly aggregated, suggesting that it may be subject to modification by the cells. These results indicate the neuronal plasma membrane may sequester amyloid assembly or otherwise stabilize amyloid aggregates and suggests that a failure to degrade Aβ1-42 may be an important component of amyloid accumulation. Supported by NIH GM07311 and NS31290.

The levels of soluble Aβ amyloid peptide in biological fluids depends both on its rate of production and its rate of removal. Human neuroblastoma cells have been shown by several groups to secrete soluble Aβ. Here we show that human neuroblastoma cells also secrete a protease that degrades Aβ. Cells were metabolically labeled with [35S]Met and the labeled conditioned medium was separated from cells by filtration. The cell-free medium was then incubated on ice or at 37°C for 24 hours, and Aβ levels were measured by immunoprecipitation, electrophoresis, and phosphorimaging. Medium incubated at 37°C contained 60%-90% less Aβ than media incubated on ice. The metalloprotease inhibitors EDTA or α-phenanthroline prevented the degradation of the Aβ. Addition of EDTA or α-phenanthroline prevented the degradation of the Aβ. This proteolytic activity was a member of the metalloprotease class. Matrix metalloproteases are generally secreted as inactive zymogens that can be activated in vivo by the organonuclear compound p-aminosalicylic acid (APMA). APMA in addition prior to the incubation step resulted in a potentiation of Aβ degradation; however, degradation under these conditions was still inhibited by α-
phenanthroline and EDTA. Moreover, general inhibitors of the other major protease classes (1,4-dichloro-2-norbornanone for serine, E-64 for cysteine, and pepstatin A for aspartic) did not inhibit Aβ degradation. This pharmacology suggests that Aβ1-42 secretes a protease of the matrix metalloprotease family that degrades soluble Aβ. To the extent that cultured cells reflect processes in human brain tissue, we speculate that inefficient proteolytic removal of soluble Aβ from brain may contribute to the etiology of Alzheimer-type amyloid formation.

612.7 MOLECULAR CHARACTERIZATION OF THE APP/ALP SUPERFAMILY. W. Wasco*, D.M. Kovacs, A. Crowley, K.M. Felsenstein and R.E. Tanzi, Laboratory of Genetics and Aging, Massachusetts General Hospital, Harvard Medical School, Boston, MA and BioTrust Squibb, Wallingford, CT.

The Alzheimer-associated amyloid β-protein precursor (APP), which was first identified in 1987, is now known to be a member of an evolutionarily conserved gene family. We have identified and isolated cDNAs encoding two mammalian APP-like proteins (APL1 and APP2), and APP-like genes have also been identified in both Drosophila and C. elegans. All of these proteins show extensive amino acid and domain homologies, and like APP, the mammalian APLs have a number of alternatively transcribed forms. Interestingly, the direct comparisons of the amino acid sequence of the APP-like genes of APL1 have demonstrated that the amyloidogenic Aβ domain is not conserved in the family and is found exclusively in APP. For our current set of experiments we have established stably transfected human h4 neurogliaoma cell lines that overexpress APP, APLP1 and APLP2. These lines are being used to characterize the subcellular localization of the APL and to compare the effects that the overexpression of one member of the family may have on the expression and processing of the others. Reagents capable of indisputably distinguishing the members of the APP-like family by immunobiochemical and immunoblot analysis are currently being developed. In addition, we have compared the tissue distribution of alternatively transcribed forms of APP and the APLs. Finally, we have examined whether the APLP gene transcripts and the specific expression of APLP alternatively transcribed domains (such as the APLP2 KPI and 12 amino acid domains) are developmentally regulated.

612.8 TISSUE DISTRIBUTION, DEVELOPMENTAL EXPRESSION, AND PROCESSING OF ALP1 ISOFORMS IN MOUSE. G. Thinkanathan*, HS Slunt*, and BS Swaroop.1. Department of Pathology, Neurology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Amyloid precursor-like protein 2 (APLP2) is a member of a larger family of proteins that includes as amyloidogenic precursor protein and APP1. APLP2 pre-mRNA undergoes alternative splicing to generate mature isoforms that encode for at least four APLP2 isoforms. We have employed quantitative reverse-transcription polymerase chain reaction assays to analyze the expression of transcripts encoding various APLP2 isoforms in adult tissues and during mouse development. In parallel, the expression of specific APLP2 isoforms is being monitored with highly specific anti-APLP2 antibodies. We have also examined the metabolism of the APLP2-751 isoform in cultured mammalian cells. In transfected CHO, COS-7, and neuroblastoma N2A cells, APLP2 is modified by chondroitin sulfate (CS) glycosaminoglycans. Using site-directed mutagenesis strategies, we have identified the site for the modification of APLP2-751. We are presently characterizing the posttranslational processing of the other APLP2 isoforms.

This work was supported by grants from the U.S. Public Health Service as well as the Adler Foundation.

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612.10


Among the transmembrane glycoproteins constituting the family of APP-like proteins, APLP2 is the nearest relative of the Alzheimer B4-amyloid protein precursor (APP). We determined the complete cDNA and amino acid sequence of rat APLP2 comprising 765 residues, and compared its tissue-specific expression and alternative splicing to APP gene expression. In APP, there are three splicing events that alter the reading frame at which, as we have previously shown, the 1-APP mRNA isoforms lacking exon 15 are ubiquitously expressed in non-neuronal cells, but not in neurons (2). In APLP2, we were able to identify two alternatively spliced exons, of which the K-exon-enriched isoform is highly expressed in neurons and thus differs in its tissue-specific expression from the APP-exon encoding APP. While no equivalent to exon 8 of APP was detected in APLP2, there is another alternatively spliced exon with a highly similarly regulated expression, that is part of that APLP2 domain most divergent to APP, L-APLP2 mRNA isoforms lacking this exon represent the major part of APLP2 transcripts in non-neuronal tissues, but are only weakly expressed in neurons (3).

Because of the similar regulation of alternative splicing of exon 15 of APP and the described APLP2 insert, and because of structural similarities of the sequences and the predicted secondary structures, a functional relatedness of alternatively spliced isoforms of APP and APLP2 is suggested. Ubiquitous expression of high levels of L-APLP2 and L-APLP2 mRNA except for neurons indicates an important function in non-neuronal cells, and is remarkable since neurons are the primarily affected cells in Alzheimer’s disease.


613.1


Developmental events that shape the nervous system are often regulated by steroid hormones. During metamorphosis in the moth, Manduca sexta, when the larval nervous system is reshaped for adult function, the hormone 20-hydroxyecdysone (20-HE) controls diverse aspects of neuronal differentiation. Little is known, however, about hormonal effects on glial cells. In the antennal lobes of Manduca, glial cells are critically important because one type, the neuron-associated glial cells, forms boundaries for developing olfactory glomeruli as a result of proliferation and migration during metamorphosis. The temporal patterns of glial proliferation is similar to the pattern of changing hormone titers, suggesting a regulatory role for hormones. We have manipulated hormone titers in vivo by injecting 20-HE into the hemolymph during different stages of metamorphosis in normal animals, the glial cell number rapidly increasing. Later, after stage 6 (of 16 developmental stages), hormone treatment became less effective at enhancing proliferation; by stage 12, when 20-HE titers are near zero, there was no significant stimulation of proliferation. Thus, the ability of cells to respond to hormone is limited to the window of time when, normally, 20-HE circulates in the hemolymph. Two other glial classes, cell-body-associated glial cells and perineurial cells also proliferate in response to 20-HE. Our results indicate that glial proliferation in the brain, like neuronal survival and growth elsewhere in the nervous system, is under the control of steroid hormones during metamorphic development.

613.2

NEUROGENESIS AND SYNAPTogenesis OF THE DEVELOPING SEPTUM AND AMYGDALA IN THE BRAZILIAN OPOSSUM BRAIN. L.J. Sassevold, J. Hjalb, J. Kiiepin*, and C. D. Jacobson, Department of Veterinary Anatomy and Neurobiology, Iowa State University, Ames, IA 50011, *Department of Neurology, Harvard Medical School, Beth Israel Hospital, Boston, MA 02115.

The Brazilian opossum, Monodelphis domestica is a small marsupial whose young are born in an extremely immature state with a protracted postnatal period of neurogenesis. We have previously shown that neurogenesis in the hypothalamus continues postnatally. We have now studied postnatal development of the amygdaloid cortex, a region comprising the amygdala (Amyg), is a high proportion of labelled cells were found in the Amyg and Sp5 following postnatal injection of BrdU during the fetal period (PN) 7 or 8. The number of BrdU labelled cells decreased with increasing age of injection. No BrdU labelled cells were present in the Amyg and Sp5 following injections on PN 12 and 13 respectively. However, a few BrdU labelled cells were labelled in the lateral septum and amygdala following PN 13 injection. To correlate neurogenesis with synaptogenesis, immunohistochemical analysis of proteins associated with synaptic vesicles, synaptic membrane, and microtubule-associated proteins during development in the Sp5 and Amyg was conducted. From 1 through 9 PN the Sp5 and Amyg are lacking immunoreactivity for the synaptic vesicle-associated proteins (SVAPs) synaptophysin and synapsin I. After 10 PN, there is an increase in immunoreactivity for the SVAPs. Tau-1 (associated with axons) immunoreactivity follows a similar pattern of expression, whereas synaptopHesin-associated protein 25 immunoreactivity is not evident until after 11 PN. In contrast, immunoreactivity for map-2 (associated with dendrites) is present in the Sp5 and Amyg from 7 PN on. These results indicate that synaptogenesis begins as neurogenesis is completed in the Sp5 and Amyg of the opossum.

613.3

CELL SIZE REDUCTION DURING NORMAL SPINAL CORD DEVELOPMENT. A. Chen* and R.D. Heathcote, Department of Biological Sciences, University of Wisconsin, Box 413, Milwaukee, WI, 53201.

Regressive phenomena play as important role in the morphogenesis of the vertebrate nervous system. For example, cell death, pruning of neuronal arborizations and synapse elimination are frequently involved in the maturation of neuronal populations. During neural tube formation in the frog Xenopus laevis, average spinal cord cell body size underwent two phases of reduction. The initial phase was characterized by a corresponding increase in cell number. To determine if cell death in cell size was caused by cell division, animals were treated with a mixture of hydroxyurea and aphidicolin (HU) to block DNA synthesis and consequently cell division. The cells of spinal cords treated at the time of neural tube closure did not decrease in size. At the earliest stages treated, showing that the first phase of reduction in cell size was the result of cell division. Subsequently, a specific population of catecholaminergic spinal cord neurones underwent an additional phase of reduction. Since catecholaminergic neurones in the peripheral nervous system can divide, we tested the possibility that this second phase of reduction might also be due to cell division. Cell division was blocked by trexitin at this time, and neurones in this phase of catecholaminergic cell size continued to decrease in size, showing that the second phase of reduction in cell size was not the result of cell division. In this case cell size reduction could be caused by two phases of apoptosis of small axons and dendrites, elimination of cytoplasmic yolk droplets or cellular secretion.

Decreases in spinal cord cell size can be separated into two phases. During the first one to two postnatal weeks the decrease in cell size can be accounted for a marked decrease in the size of spinal cord cell bodies. During the subsequent one to two days, at least some differentiating cells undergo a greater than twofold decrease in size that is independent of cell division.

613.4


In adult lizard, CNS repair and regeneration processes are apparently carried out by cells originating from specific areas (i.e. sulci) of the ventricular wall. Little is known on these structures and on their embryogenesis in reptiles. We have thus begun a study on the development and differentiation of ependymal cells in Gallia galli, a lizard indigenous to the Caribbean Islands. By using EM and IHC studies we found that these cells are lined with an undifferentiated pseudostriatified columnar neuroepithelium where all cells are Vimentin positive. Between E33 and E37, Vimentin immunoreactivity decreases while GFAP appears in ependymal cells and vestiges of ependymal cells and microtubules become evident in the cytoplasm of ependymal cells. No apparent changes were observed in cell size or cell shape. We observed that glial cells begin to exhibit phenotypic differences. During this period three types of ependymal cells become distinguishable: columnar cells, subependymal cells and ependymal cells, which line the ependymal cell layer. These cells mature into radial glial cells (which in lizard is present also in adults), others into tanyctyes, like cell exhibiting microtubule rich processes. Possibly the processes of the first correspond to the GFAP rich processes while those of the latter correspond to the Glutamine synthetase rich processes. A comparison with rat ependymal development was also carried out.

Studies are now in progress aiming at identifying the factors that result in new cell multiply after CNS lesions in the adult lizard.

References
613.5


Although it has long been established that neocortical development occurs in a rostral to caudal direction, much less is known about the time course of events, which has not been elaborated in many species or regions of cortex. To this end we wished to establish when cells destined for specific layers of ferret somatosomatic cortex are generated. By means of the well-studied visual cortex and their association to intrinsic cortical connectivity. Timed-pregnant ferrets were injected IV with bromodeoxyuridine (BrdU) on embryonic days (E) from E22 to E38 and on postnatal days (PND) 1 and 4. Cortical tissue was collected from ferrets kits after various survival times, ranging from PND 1-60. As expected, a progressive sequence of inside-out maturation occurred in the genesis of cortical layers. Neurons labeled by BrdU migration into the ferret cortex and were found in the subplate up to PND 14. Injects of BrdU on E33-35 labeled neurons found in layer 4 in the mature cortex; and on E38 labeled neurons found in layer 2. Several features of the developing somatosomatic cortex differed from previous observations in the visual system. We observed a few neurons distinctly populating the cortex generated postnatally. Although occasional BrdU-positive cells were seen in the cortex after injections on PNDs 1 and 4, they were highly dispersed and not definitively localized in any particular layer. In addition, by PND 1, neurons residing in all cortical layers, including layer 2, were observed in the cortical plate. Therefore, experiments with fluorescent tracer reveal that many neurons are still migrating to the somatosomatic cortex during the first week after birth, elements of the laminar scaffolding are in place at PND 1. These findings confirm the notion of a rostral to caudal gradient of cell production and suggest that elements of ferret somatosomatic cortex development may occur up to a week prior to visual cortical development.

613.7

EFFECTS OF INTERLEUKINS ON NEURONAL AND OLIGODENDROGLIAL LINEAGES IN EGF-GENERATED CNS STEM CELL CULTURES: R.E. Gross*, M.F. Mehier, I. Santschi, and J.A. Kessler, Deps. of Neurology and Anatomy & Neurology, Albert Einstein College of Medicine, Bronx, NY 10461

Recent experiments have begun to demonstrate that various neurotrophic cytokines play an important role in the survival and maturation of neural stem and progenitor cells along the three neural lineages. We have previously reported that certain interleukins (IL) affect neuronal maturation of immortalized and primary neural cell lines, and in other studies, have identified cytokines for neurons in early development in vivo. Here we extend our studies of the role of ILs in neural development by investigating their effects on non-immortalized neural stem cell lines. EGF-dependent neural stem cells were generated from embryonic or adult rodent brains using a method developed by Reynolds & Weiss (1992). Neurons were dissociated, washed twice, and cultured on poly-ornithine coated coverslips in serum-free medium (SMF) with TGfβ in the presence of various ILs, followed by immunocytochemistry for markers of the three neural cell lineages. Control cultures contained a large number of map2-positive neurons and a relatively small number of myelin basic protein (MBP) - positive, highly active oligodendroglial progenitors (M2 and I1). IL-4, IL-2 and IL-12 and IFN-γ had a marked stimulatory effect on both the number and length of neuritic branches of a subset of map2-positive neurons without affecting the total number of neuron generated. Thus, certain neurotrophic cytokines may affect maturation of a set of neurons without influencing survival or proliferation. In contrast, cytokines present in the presence of cytokines contained four- to five-fold greater numbers of MBP-positive oligodendrocytes, suggesting a role for interleukins signaling through non-gp130 receptors in proliferation, survival or differentiation of cells of the oligodendroglial lineage.

613.8


Neurogenetic regulation has been extensively studied in insect brain development, including cerebellar granule and sympathetic neurons. In contrast, mechanisms governing the complex cerebral cortex in which multiple neuronal lineages, remain undefined. Do common signals regulate cortical precursors? Preliminary Adenylate Cyclase Activating Peptide (PACAP, bPP) action on other lineages, PACAP (109) inhibited cortical (bH)yamine incorporation 50%, reflecting a similar decrease in the percentage of neuroblasts entering the mitotic cycle. Inhibition was observed even in the presence of mitogenic stimulation elicited by serum, insulin, EGF and FGF. Other VIP family members, including PHI, secretin and VIP, were without effects at equimolar concentration, indicating that PACAP activity is highly specific. However, since VIP was inhibitory at higher doses (10), the peptide apparently acts via PACAP type I receptors. To begin defining mechanisms, we examined second messenger pathways: cAMP agonists, including forskolin, DI-AMP and IBMX, reproduced PACAP's effects. Further, the peptide elicited an 1.5-fold increase in cAMP.

In addition to inhibiting mitosis, the peptide increased trkB immunoreactivity by 30% and neurite outgrowth by 80%, suggesting that PACAP blockage of proliferation induced neuronal differentiation. In sum, while the PACAP ligand-receptor cAMP pathway regulates multiple precursors, effects are lineage-specific. Endogenous PACAP may play a critical role in regulating progress of cortical neuroblasts through the ontogenetic sequence.

613.9

LINEAGE OF SUBPLATE (SP) AND CORTICAL PLATE NEURONS IN FERRET STUDIED WITH AN ALKALINE PHOSPHATASE (AP) RETROVIRAL LIBRARY: C. Wahl*, I. Cepko*, C. Reid*, and L. Jiang. Neurobiology, Beth Israel Hospital, and Programs in Neuroscience and 2Biological and Biomedical Sciences, Harvard Medical School, Boston, MA 02115

Since the ferret allows analysis of early cortical development, we produced an amphotropic retroviral library that encodes AP and contains up to thousands of DNA tags for cell lineage analysis. Cortical cells were labeled by injections at E27- P0, and proceeded with immunocytochemistry and clonal analysis by polymerase chain reaction (PCR) (Wahl & Cepko, Science 255:432, 1992) at P17. As expected, early injections (E27 and E29) labeled deep layer cells, including subplate neurons, in addition to mitotic neurons (bromodeoxyuridine (bODU) and 5-bromo-2-deoxyuridine (BrDU) and IFN-γ had a marked stimulatory effect on both the number and length of neuritic branches of a subset of map2-positive neurons without affecting the total number of neuron generated. Thus, certain neurotrophic cytokines may affect maturation of a set of neurons without influencing survival or proliferation. In contrast, cytokines present in the presence of cytokines contained four- to five-fold greater numbers of MBP-positive oligodendrocytes, suggesting a role for interleukins signaling through non-gp130 receptors in proliferation, survival or differentiation of cells of the oligodendroglial lineage.

613.10

PROLIFIC EXPRESSION OF A TUMOR SUPPRESSOR GENE, APC IN GLIAL CELLS: Ratan V. Bhat*, Karen J. Act and Jay M. Baraban, Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Tumor suppressor genes act as brakes to control excessive cellular proliferation and therefore could play a key role during development. Adenomatous Polyposis Coli (APC) gene product. (APC) is a tumor suppressor gene that is mutated in a familial type of colon cancer. In previous studies, we found high levels of APC mRNA during ON development. Immunoblot of brain extracts confirmed the presence of APC protein (310 kD). To identify the cell types expressing APC protein during development, we performed immunohistochemical and biochemical studies on brain sections from E19-P30 rats. Prominent APC staining was present in olfactory and olfactory precursors as early as E19 in the brain of E19 rat. APC immuno-staining was observed in olfactory/oligodendrocytes of cerebellar white matter in immature mouse. In the cerebellum, APC staining was seen in the Bergmann astrocytes and ciliated satellite oligodendrocytes in the granule cell layer. The high levels of APC expression in astrocytes and oligodendrocytes indicates that this tumor suppressor gene may have a role in controlling glial proliferation.
613.11 A BRAIN-SPECIFIC ACTIVATOR FOR CYCLIN-DEPENDENT KINASE 5. L.-H. Tsai* and E. Harlow, Molecular Oncology, MGH Cancer Center, Charlestown, MA 02129.

Cyclin-dependent kinase 5 (cdk5) shares about 60% identity at the amino acid level with both cdc2 and cdk2, kinases that play key roles in cell cycle progression. Despite the fact that cdk5 is similar in structure to cdk2 and cdk2, it displays properties distinct from those kinases. First, cdk5 is expressed in a tissue-specific manner, being highest in adult nervous system and low or undetectable in embryos and adults. Second, we have detected kinase activity of cdk5 only in post-mitotic cells of neuronal origin in brain. Finally, cdk5 does not form an active kinase complex with the conventional cyclins. We observed a 35 kd protein that was associated with cdk5 from primary neuron cultures containing active cdk5 kinase activity. A CDNA for p35 has recently been cloned. Expression of p35 in human cultured cells line activated both the endogenously as well as exogenously overexpressed cdk5. Coexpression of p35 with a dominant negative version of cdk5 in these cells abolished the ability of p35 to activate cdk5. Cdk5 appears to be the only member of the cdk family proteins that is readily activated by p35. Therefore, p35 serves as a specific activator for cdk5. Surprisingly, p35 displays no sequence homology to any existing cyclin molecules. Northern analyses indicated that p35 is only expressed in brain. Among the central nervous system p35 is most highly expressed in the forebrain including cerebral cortex and thalamus, and it is not detectable in the hind brain or spinal cord.

613.12 CELLULAR DISTRIBUTION OF Dlx2 mRNA IN THE DEVELOPING BASAL GANGLIA. M. Ding, L. Robel, A. James, and P. M. Vaccarino*. Child Study Center, Yale University School of Medicine, PO Box 3333, New Haven, CT 06520.

The acquisition of a morphological and functional identity of different areas of the nervous system may be regulated by specific combinations of transcription factors. In the developing mammalian nervous system, expression of a given transcription factor is often associated with expression of other transcription factors whose expression is functionally relevant to the developing area. A recent study of the basal ganglia, a region of the brain that is responsible for the control of voluntary movement, revealed that the expression of homeobox genes of the Dlx family is restricted to the basal telencephalon, the anlage of the basal ganglia. However, the exact cellular location of these genes is presently not known. Cells obtained from the ventricular layer of the basal telencephalon at the embryonic day 13.5 and grown in primary culture express Dlx1, Dlx2, Dlx3 and Dlx6, as they do in vivo. In contrast, primary cultures of cells from the dorsal telencephalon do not express the Dlx genes. The time course of expression of Dlx2, Dlx3 and Dlx6 mRNAs peaks at 3-5 days in vitro and is protracted during the entire period of neuronal differentiation and maturation. Dlx2 mRNA, identified by in situ hybridization, is localized in young neurons, immunoreactive for the microtubule-associated protein MAP1B, and, to a lesser extent, in more mature neurons, which contain MAP2 immunostaining. Nestin-positive stem cells and GFAP-containing astrocytes never expressed Dlx2 mRNA. Blocking Dlx2 gene expression by means of antisense oligonucleotides resulted in lower numbers of MAP1B- and MAP2-immunoreactive cells and in a dramatic change in the morphological characteristics of the cultures. Nestin and GFAP immunoreactivity were unchanged by the antisense Dlx2 treatment. These data suggest that the expression of genes of the Dlx family may control important characteristics of the non neuronal phenotype in the developing basal ganglia.

613.13 TREATMENT WITH BFGF INCREASES THE EXPRESSION OF Otx2, Dlx2, and Dlx5 HOMEobox GENES IN PRIMARY CULTURES OF CELLS FROM EMBRYONIC DAY 13.5 RAT TELENCEPHALON. L. Robel, M. Ding, A. James, A. Simpence, J. Leckman*, and P. M. Vaccarino. Child Study Center, Yale University School of Medicine, New Haven, Connecticut 06520 USA.

The characterization of the domains of expression of homeobox genes specifically expressed in the mammalian forebrain (Dlx1, Dlx2, Dlx3, Dlx5, Emx1, Emx2, Otx1, Otx2) led to the hypothesis of its early regionalization. So far the role of these homeobox genes in cell specification has not been clearly demonstrated. To address this question, we studied the effect of basic fibroblast growth factor (BFGF) on the expression of these homeobox genes in cultures of cells obtained from embryonic day 13.5 rat telencephalon. In this in vitro preparation, stem cells multiply and then differentiate into aspartate-, glutamate- and GABA-containing neurons as well as in glial cells. We have previously shown that BFGF treatment leads to a 3-fold increase in the number of glutamate-containing neurons. Using an RNA probe protection assay we observed an increase in the expression of Otx2, Dlx2 and Dlx5 at 3 and 5 days in vitro, corresponding with active neuronal differentiation in the cultures. There was no such increase at day 12, after completion of the neurogenesis. Otx2 is expressed in the ventricular zone from E8.5, whereas the Dlx genes are expressed in the subventricular and mantle zones within neurons (Ding et al., Neurosci. Abstr. 1994). These results suggest that Otx2 genes mediate the effects of BFGF on neuronal differentiation through a concerted expression of downstream genes, including Dlx2 and Dlx5.

613.14 THE MOLECULAR GENETICS OF NEURONAL GROWTH CHARACTERIZATION OF THE Tau α-TUBULIN PROMOTER IN DEVELOPING TRANSGENIC MICE. A. Glover, A. Speedman, J. Toms, E. Chan, and F. D. Miller. Centre for Neuronal Survival, Montreal Neurological Institute, McGill University, Montreal, Canada.

The role of the α-tubulin gene is regulated as a function of neuronal growth in both developing and mature mammalian neurons. We have generated transgenic mice carrying 1,100 nucleotides of the upstream, putative Tau 1 promoter region linked to a nuclear [γ-galactosidase reporter gene. Developmentally, expression of the transgene appears early during embryogenesis and is coincident with neurogenesis. Expression of the transgene is limited to the transgenic nervous system, and is first seen in the spinal cord, brain, trigeminal ganglia, facio-acoustic ganglia, and in the heart at E9.5, and in the retina at E12.0. This expression remains high during neuronal maturation and is subsequently downregulated following neuronal maturation, as indicated both by X-gal staining and by Western blot analysis. However, in the adult the olfactory epithelium, a region of ongoing neurogenesis, expression remains elevated. Thus, sequences exist within this promoter region which are responsible for coupling gene expression to neurogenesis and neuronal growth exist during development. We now delineate regions within this 1.1 kb fragment of the α-tubulin promoter, to see which regions are responsible for conferring tissue and developmental specificity upon the expression of the transgene.

613.15 ANALYSIS OF CIS-ELEMENTS IN THE Tau α-TUBULIN PROMOTER. D. Rogers, N. Laferreri, A. Glover, D. Brown*, F. Miller. Centre For Neuronal Survival, Montreal Neurological Institute, McGill University, Montreal, Canada. # Dept. Biology, University of Ottawa, Ottawa, Canada.

The Tau α-tubulin gene is a neuronal specific isotype of α-tubulin which is expressed as a function of neuronal growth. We have previously utilized 1 kb of 5' upstream promoter region to drive the expression of the lacZ reporter gene in transgenic mice. The pattern of expression was found to mimic the spatiotemporal expression pattern of the endogenous gene. Furthermore, we have previously demonstrated that this promoter region is capable of driving lacZ expression in P19 embryonal carcinoma cells which are induced to adopt a neuronal fate using retinoic acid. To characterize the relevant cis elements, we have developed in the developing in the expression of Tau α-tubulin, deletion mutants of the Tau α-tubulin promoter were generated. Mutant promoters were assayed for their ability to drive lacZ expression in P19 embryonal carcinoma cells which can be induced to adopt a neuronal fate using retinoic acid. The effects of deletion of specific cis-elements on expression of the reporter gene in P19 cells was determined.

613.16 BIRTHDAYS AND SURVIVAL AFTER ABDOMINAL NEUROCHEMICALLY DEFINED SUBSETS OF TRIGEMINAL GANGLION CELLS. P. A. Whiteside, C. L. Macdonald, and F. R. Hoagland. Dept. of Anatomy, Medical College of OHIO, Toledo OH 43669.

Trigeminal (V) ganglion cells with different neurochemical phenotypes have different birthdates, which are affected differently by neonatal axonal transaction. This study attempted to determine whether trigeminal ganglion cell birthdate and neurochemical phenotype were correlated and if these variables could be used to respond to neonatal axonal transaction. Immunocytochemistry, previously used to determine the birthdates of V ganglion cells recognized by antibodies directed against the retinoic acid receptor (RANK), calcitonin gene-related peptide (CGRP), substance P (SP), and those that bound the lectin Bandeiraea simplicifolia (BS-1). The percentages (normalized to equal 100%) of NF-positive ganglion cells born on E-9.0, 10.5, 12.5, 13.5, and 14.5 were 0.9%, 27.3%, 30.3%, 37.0%, 4.2%, and 0.3%, respectively. The respective values for CGRP-positive ganglion cells born on E-9.0, 10.5, 12.5, 13.5, and 14.5 were 0.2%, 2.3%, 3.9%, 51.5%, and 42.1%, respectively. Those for SP-positive neurons were 0.4%, 0.2%, 16.0%, 65.0%, and 15.4%, and those for BS-1-positive ganglion cells born on E-9.0, 10.5, 12.5, 13.5, and 14.5 were 0.1%, 2.4%, 10.9%, 7.2%, 70.9%, and 8.6%, respectively. A significant correlation was found. The percentage of CGRP-positive ganglion cells born on E-9.0 was 2.3% in the axotomy group, which was significantly lower than the percentage of CGRP-positive ganglion cells born on E-9.0 in the control group, which is 27.3%. These findings do not indicate a strong relationship between cell birthdate, phenotype, and survival after neonatal axonal damage. Supported by NS 28848 and DE 07734.
613.17 ABLATION OF CEREBELLAR ASTROCYTES IN POSTNATAL TRANSGENIC MICE Catherine L. Delaney, Michael Bremer, and Albania Mussinat Neuroscience Training Program and Dept. of Pathobiological Sciences, Univ. of Wisconsin-Madison, Madison, Wisconsin 53706

Stroke Branch, NINDS, NIH, Bethesda, Maryland 20892

To study the role of astrocytes during CNS development, a transgene was designed with the ability to ablate astrocytes in a developable manner. The transgene consists of the coding region for the herpes simplex virus thymidine kinase (hsv-tk) under the control of the human glial fibrillary acidic protein gene promoter. The hsv-tk is innocuous, but converts the antiviral agent ganciclovir (ganc) with high potency into a toxic analog that interacts with replication in proliferating cells. Treatment of transgenic mice during the first postnatal week with ganciclovir, with evaluation at 19d postnatal, revealed markedly behavioral abnormalities and ataxia; clamped their feet when suspended, and had difficulty standing upright to obtain food. Histological examination revealed disrupted astrocyte development, particularly in the cerebellum, with marked secondary effects on other cell types. Cerebellar defects included an overall reduction in size and disruption of the normally well defined cellular layers. The radial glia were disordered, and there was an apparent defective myelination. The molecular and granule cell layers were greatly reduced in size. There was marked depletion of granule cells, and Purkinje cells were ectopically distributed in the molecular layer. These effects were more severe in animals treated 1d postnatal versus treatment at 5d. These results suggest a critical role for astrocytes in cerebellar development.


The mouse genetic mutation meander tail (gene symbol mea) results in the near total ablation of granule cells in the anterior lobe of the cerebellum. How the mea gene results in granule cell reduction remains unknown. Two approaches were used to determine whether the action of the mea gene is specific to anterior lobe granule cells. First, we find by estimating granule cell number in the anterior lobe, a significantly larger (nearly 90%) reduction in granule cell number was observed in the mea mea+/- genotype compared to the mea+/- and mea+/-+/- genotypes. Second, we studied intracellular calcium in two types of interspecies murine chimera (homozygous mea mea mice compared to heterozygous controls. EGF+bFGF treatment of mea+/- to mea+/-+/- cells from meander tail embryos labeled immaturecally at 19d postnatal with a streptavidin specific mouse neuronal antigen (Mullen and Chochick, 1989). We find that both cell types are labeled by this method but only the mea+/-+/- cells were labeled with the meander tail mutation. Thus the mea mea mea+/-+/- cells are found in both the anterior and posterior lobes. Additionally, the percentage of cells that are granule cells is reduced in the cerebellar granule cell layer in anterior and posterior lobes as well as in hippocampus, olfactory bulb granule cells, and in cerebellar molecular layer interneurons. Cerebellar granule cells that are genetically meander tail are found in both anterior and posterior lobes. However, the percentage of mea cerebellar granule cells is markedly less than the percentage of mea cells found in other neuronal populations including the cerebellar molecular layer interneurons. These results suggest that while the mea gene directly affects overall granule cell survival, there are extrinsic factors that promote the survival of mea+/- granule cells in the anterior lobe. Support: NS23475 to DG.

613.20 EPIDERMAL GROWTH FACTOR (EGF), TRANSFORMING GROWTH FACTOR-alpha (TGF-alpha) AND BASIC FIBROBLAST GROWTH FACTOR (FGF) DIFFERENTIALLY INFLUENCE NEURAL PRECURSOR CELLS OF THE MOUSE EMBRYONIC MENSECEPHALON. J. Santa-Ulalia and L. Gravellano. Departamento de Biologia Molecular, Instituto de Biotechnologia, UNAM, Apdo. Postal 510-3, Cuenavaca, Morelos 62271, Mexico.

The molecules generally known as growth factors are key elements in the process of neural cell differentiation. In this report, we examined the effects of classical mitogens on neural precursor cells, by culturing mouse cells of the embryonic (13.5 days post coitum) menencephalon and treating them with EGF, TGF-a, and FGF. Neuro Growth Factor (NGF) and Transforming Growth Factor (TGF-ß) were added in the initial cultures. The results showed that EGF, TGF-a or FGF but not NGF-ß induced general proliferation of the cultured cells, followed by the formation of colonies. Colonies maintained in the presence of EGF or TGF-a produced a neurophilic factor, while the population that was treated with FGF was not. The mea+/-+/-+/- cells are found in both the anterior and posterior lobes. The percentage of cells that are granule cells is reduced in the cerebellar granule cell layer in anterior and posterior lobes as well as in hippocampus, olfactory bulb granule cells, and in cerebellar molecular layer interneurons. Cerebellar granule cells that are genetically meander tail are found in both anterior and posterior lobes. Additionally, the percentage of cells that are granule cells is reduced in the cerebellar granule cell layer in anterior and posterior lobes.

614.2 MIGRATION OF LHRR NEURONS IN OLFATORY ENSLANTS: EFFECTS OF SERUM-FREE MEDIA, TETRODOTOXIN AND DEPOLARIZATION. S. M. Fuesko* and S. Wray Lab of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

We have previously shown that embryonic mouse olfactory explants maintain large numbers of LHRR neurons which emerge and migrate away from the olfactory pit in directional tracks along a sub-layer of peripherin positive fibers. To continue our study of the mechanisms guiding the neurophilic migration of LHRR neurons, we examined the migratory properties of LHRR neurons cultured in serum-free media (SFM). We also examined whether spontaneous electrical and synaptic activity and/or depolarization play a role in the movement of LHRR neurons. Olfactory explants from E11.5 mouse embryos were cultured in SFM for 1-7 days. Immunohistochemistry stained indicated that compared to explants grown in the presence of serum, SFM had no significant effect on LHRR cell numbers (25-50% of the total LHRR population was typically maintained) or on LHRR cell migration as determined by emergence, directionality and distance that cells migrated away from the olfactory pits. Inhibition of spontaneous electrical and synaptic activity by continuous incubation with tetrodotoxin (10-5M) from day 2-7 in culture had no significant effect on either LHRR cell number or the ability of cells to migrate. Similarly, depolarization by incubation with potassium (20 mM) had no significant effect on LHRR cell migration. Therefore, we propose that interactions at the cell surface which are independent of spontaneous electrical activity and/or depolarization influence secretion from embryonic LHRR cells.
614.3  GABA and NGF INDUCE CHEMOTAXIS IN CORTICAL NEUROBLASTS. T.N. Behar*, H.T. Tran, and J.L. Barker. Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD. 20892

Chemotactic and chemokinetic migration of acutely dissociated embryonic cortical neurons in serum-free defined medium from 14 to 19 day-old rat embryos (E12-E15) was analyzed in vitro using a chemotaxis chamber.

Embryonic cells migrated toward γ-amino butyric acid (GABA) beginning at E15, indicating cells generated in the ventricular zone migrate towards GABA. In contrast, NGF-induced migration was not detected until E17, suggesting cells generated in the subventricular zone respond to NGF. A modified checker-board analysis revealed that micromolar GABA exerted a chemokinetic effect on cells (increased random motility), while PM NGF and low GABA concentrations (M) were predominantly chemotactic, inducing cells to migrate along a chemotactic gradient. Immunolabeling showed that the majority (>95%) of migrating cells expressed neurofilament protein (NF) and hence, were postmitotic neurons. The chemotactic effects of GABA were mimicked by diethylbarbituric acid (DEB) and GABA mediated motility via both GABA_A and GABA_B receptor proteins. These results demonstrate that both GABA and NGF are capable of directing the migration of newly generated neurons during early cortical development.


Following their birth in olfactory placode, genodatrophins releasing hormone (GnRH) containing neurons migrate across the developing cribiform plate and form a dispersed population in the mammalian basal forebrain. To study the migration of these neurons we are utilizing a paradigm to visualize cell migration in vitro, in tissue slices that maintain the structure and contact of the nasal cavity with the forebrain. On day 15 and 16 of gestation rat embryos were dissected, and the heads were embedded in agarose and cut by Vibratome into 300 micron slices. Individual cells were randomly labelled by briefly immersing the slices in the carbocyanine dye, Dil. Cells were selected for injection sites and rates of cell movements were analyzed throughout the rostral-caudal extent of the GnRH cell migration pathway. Characteristics of cell movements differed depending upon location along the pathway. Cell movements in the nasal cavity involved fewer turns than in the basal forebrain. These studies raise the possibility that there are constraints on the ability of cells to move in selective portions of the migration pathway from the nasal cavity to the basal hypothalami. Supported by PHS grants HD05515, MR Core Grant HD-04147, and the DMR-MA.

614.6  THE VENTRALLY GENERATED "U-SHAPED" GROUP OF CHOLINERGIC CELLS IN EMBRYONIC RAT SPINAL CORD EXPRESS NADPH-DIAPHORASE AND MIGRATE DORSALLY IN VIVO AND IN VITRO. P.E. Phelps*, R.P. Barber, R. Wett, and J.E. Vaughn. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Most neuronal migration is thought to be directed radially from the ventricular zone (VZ), but members of the "U-shaped" group of cholinergic neurons, first detected around the ventral VZ, appear to move dorsally within the spinal cord between E15 and E18. Some of these cells translocate relatively long distances to the dorsal horn, and others, only short distances to surround the central canal. Both dorsal horn and central canal cluster ChAT-immunoreactive cells co-express NADPH-diaphorase, in contrast to the dorsal horn of the "V-shaped" group that are composed of only these immature neurons. As a first step in analyzing this dorsal migration experimentally, 300-400 μm slices of E16 cervical enlargement were cultured for periods encompassing in vivo migration times. Preliminary data suggested that diaphorase-labeled cells of the "U-shaped" group had moved dorsally in a relatively histotopic pattern before differentiating. Thus, these in vitro data provide additional information that the "U-shaped" group of cells have a unique migratory pattern from the ventral VZ into the dorsal horn. Supported by NIH grant NS 18858.

614.7  CALCIUM ION INFLUXES THROUGH THE PLASMA MEMBRANE TRIGGER SPONTANEOUS INTRACELLULAR CALCIUM OSCILLATION IN MIGRATING NEURONS. H. Komuro* and P. Rakic. Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510.

Recently, we found that Ca2+ influx, regulated by a combination of voltage- and ligand-gated channels, is essential for extension of the leading process and translocation of cell soma during neuronal cell migration (Komuro & Rakic: Science 257: 806, 1992; 268: 95, 1993). Furthermore, we reported that migrating neurons exhibit transient elevation of intracellular Ca2+ (Ca2+-influx) and basal oscillation of intracellular Ca2+ (Ca2+-oscillation) (Komuro & Rakic; Soc Neurosci Abstr 19: 34; 1993). Here, we study possible cellular mechanisms underlying spontaneous intracellular Ca2+ oscillation in migrating neurons. Rectangular pieces (100-200 μm) of cerebella from 3-7 day-old CD-1 mice were placed on poly-L-lysine- and laminin-coated Petri dishes containing 1 ml serum-supplemented culture medium. After 2-3 day incubation, cells were loaded in serum-free, defined medium with 1.3 mM Fluo-3/AM for 30-60 min at 36 °C. After rinsing and an additional 1-3 h postincubation in defined culture medium, granule cell migration from microexplants was examined using confocal laser microscopy. Changes in the rate of movement of the cells and the levels of intracellular Ca2+ were directly recorded in single optical sections collected at 1-60 sec intervals for up to 70 min. Migrating granule cells exhibited unidirectional movement, and spontaneous oscillations of intracellular Ca2+ levels at a frequency of 10-20 times per hour. The duration of each elevation lasted 1-2 min; the amplitude as indicated by Fluo-3 fluorescence ranged between 5-50% of resting levels. Reduction of extracellular Ca2+ concentrations eliminated the spontaneous Ca2+-oscillation and cessation of cell movement. Furthermore, the addition of antagonists to either N-type Ca2+ or L-type Ca2+ channels abolished the frequency and amplitude of Ca2+ oscillations and decreased the rate of cell migration. These results suggest that spontaneous oscillation of intracellular Ca2+ levels may be triggered by Ca2+ influx through a combination of N-type Ca2+ channels and NMDA receptors. Furthermore, the assembly and disassembly of neuronal cytoskeletal elements associated with migration may be controlled by the periodic increase and decrease in intracellular Ca2+ levels. (Supported by NS22807)


The avian forebrain is unique in its potential for extensive repair following brain injury, from ependymal/blood vessel zones (Tubingenz) cells crossing the ventral surface of the lateral ventricle. New neurons migrate from the SA along radial glial guides neurites and then migrate into the neostriatum via the longaxon fiber. The migration of these neurons varies with the extent of injury. In the cavitary sections of both adult finch and canary brain, NG-CAM/E9 was found throughout the ventricle and its adjacent territories, but very low (less than 1%) in the control brain. In contrast, the ependymoglia and ependymal/radial fibers upon which the neurons migrated were never found to express NG-CAM/E9, in vivo or in vitro. Addition of anti-NG2 Fab to these cultures yielded a dose-dependent inhibition of neuronal outrow from the parent explant. In addition, neurons exhibited the outgrowths at the time of antibody addition exhibited process retraction, cell rounding, and in some cases, late death. Migrating neurons responded to anti-NG2 with increased cell viability in cytocinotic cultures, as viewed by laser-activated confocal microscopy. Ependymoglia generated the antibody and the migration of new neurons from the adult avian brain were blocked by the expression of NG-CAM/E9, and their adhesion to a heterologous ependymal/radial cell receptor. Furthermore, the viability of new neurons may be regulated, directly or indirectly, by NG-CAM/E9 activation-dependent, calcium-mediated signaling processes. Supported by NINDS R29NS29813, NEI-05285, The Mathers Charitable and Lookout Foundations, The Hirsch Trust and American Paralytic Association.

Luteinizing hormone-releasing hormone (LHRR) neurones originate in the epithelium of the medial olfactory pit and migrate into the brain along a migration route formed by the vomeronasal and terminal nerves. This migration route, rich in neural cell adhesion molecule (NCAM), is formed before the LHRR neurones are first detected. In 26-31 day old embryos, NCAM-immunoreactive cells were seen in the epithelium of the medial olfactory pit and emerged to form a cellular aggregate below the rostral forebrain. Antibodies to a polyclonal form of NCAM (PSA-NCAM) showed an immunoreactive staining in the basal meninges at these ages. In 41-42 day old embryos LHRR-immunoreactivity was detected in some cells among the cords of NCAM cells in the nasal meninges, just outside of the epithelium of the olfactory pit. LHRR and NCAM were not co-localized. Many more LHRR cells were seen in the ganglion terminal of the terminal nerve and along the caudal margin of the cellular aggregate below the rostral forebrain and at the basal lamina of the medial forebrain, caudal to the developing olfactory bulb. A few LHRR cells were seen in the medial basal forebrain and NCAM was present in all parts of the migration route. The PSA-NCAM, in association with LHRR cells, was seen in fasiccies in the nasal meninges, in the ganglion terminal, along the medullar margin of the cellular aggregate but not in the cellular aggregate below the rostral forebrain.


Neuropileal cells continue to migrate from the spinal cord into the periphery after neural crest emigration is completed. Cells emigrating at st. 25 differentiate into dorsal root ganglion cells and melanocytes. Do spinal cells continue to migrate away from the cord at still later stages? If so, what is the developmental fate of these cells? Migration of neuropileal cells in the spinal cord was studied in st. 29-30 chicken embryos. Cells were labeled with Dil injected into the central canal of cultured spinal segments. Labelled cells left the spinal cord through the dorsal and ventral spinal boundary, adjacent to the roof plate and through the floor plate, and migrated into the meningeal anlage. To determine where in the spinal cord were migrating, we made small injections of fluorescent dextran into the floor plate of st 29 cultured spinal segments. After 12 hours, labeled cells had migrated into the meninges. Further evidence of the fate of these cells was obtained from chimeric embryos in which quail spinal cord was transplanted into chick hosts at stage 25-26. By st 31 to P3, quail cells were present in pia, arachnoid and dura. These cells were not seen in embryos in which neuroepithelium was labeled with Dil at stage 25-27 and embryos allowed to develop for only 24 hr, suggesting that emigration of meningeal cells occurs after st 27.

These results suggest that in avian embryos, spinal cord cells emigrate through the roof plate and the floor plate and contribute to the anlage of the developing pia, arachnoid and dura surrounding the spinal cord. Supported by the NIH.


The early expression of the cholinergic phenotype in sympathetic neurones appears to be species dependent. Moreover a dual phenotype status during the noradrenergic/cholinergic transition in rodent has been electrophysiologically and biochemically immunocitochemical evidence, indicating co-expression of tyrosine-hydroxylase and choline-acetyltransferase (CHAT) in avian sympathetic neurones. We investigated dissociated cells from the superior cervical sympathetic ganglia (SCG) of piglet days 10-13 from the presence of dopamine-β-hydroxylase (DβH) and CHAT using immunocytochemical methods, in control conditions, continuous stimulation with high K+ or in coculture with splenocytes. Four types of cells were distinguished: negative cells (non-neuronal cells), DβH+ or CHAT+ positive cells, double positive cells (DβH+ & CHAT+). After 2 days in culture the ratio of CHAT+ single positive cells was 0.15 ± 0.01, indicating the predominance of the adrenergic phenotype. After 8 days in control medium the ratio increased to 0.30 ± 0.02 indicating a shift to the cholinergic phenotype. After 8 days in high K+ medium, the ratio was 0.15 ± 0.03 while coculture of the neurones with splenocytes induced a shift in the ratio to 0.41 ± 0.03. For the double positive cells, after 8 days in culture, the percentage of the DβH+ & CHAT+ cells in total neuronal cells was 8.4%, 7.4% and 17.5% in control, high K+, and co-culture with splenocytes conditions respectively. We conclude that the fetal pig neurones are predominantly adrenergic and that during a certain culture period, catecholamines and acetylcholine biosynthesis enzymes are present in the same mammal sympathetic neurones.


Genetic deletion of NCAM-100 results in a smaller olfactory bulb (OB) and morphological alteration of the subependymal layer (SEL), the latter being the source and migration pathway for OB precursor cells. The mutant mice also showed a nearly complete loss of NCAM-associated polysialic acid (PSA) that is normally expressed in these cells. The present study was intended to determine the contribution of PSA to the phenotype of the SEL. Newborn mice received an intracranial injection of endonucleaseinase N (endo N), which completely and specifically degrades PSA, and the distribution of precursor cells was examined at P1 and P7. At P1, which is prior to the appearance of the mutant phenotype in the SEL, endo N injection did not produce any morphological alterations. That is, the precursor cells tended to be positioned in caudal SEL. At P7, however, the mutant and endo N-treated animals showed the same clear perturbation: the number of OB precursor cells remaining in the caudal part of the layer increased from less than 20% to over 50%, and there was a corresponding decrease in cells that have arrived at the OB. Pulse-labeling of the SEL cells at P1 revealed an arrest in migration of cells in the caudal SEL. By contrast, there were no detectable changes in cell proliferation or death. These results provide direct evidence that the NCAM-100 mutation causes a decrease in tangential cell migration in the SEL, and that this perturbation is due to the corresponding loss of PSA.


A subpopulation of neuropileal cells in the spinal cord migrate into the periphery after neural crest emigration is complete to develop as DRG cells and melanocytes, formerly thought to be late neural crest derivatives. Could these spinal cells develop other neural crest phenotypes if they encountered the environment that earlier crest cells find? This was tested by placing the neuropileal cells into crest migratory pathways at various developmental stages. Cells from dorsal or ventral halves of st 26 quail spinal cords were dissociated and placed into chicken hosts. Approximately 100 - 200 cells were injected under the skin at the dorsolateral margin of the neural tube in st 16-19 or st 22-24 embryos. Injected embryos were allowed to develop for 2 - 10 days and quail cells were identified using a quail-specific Mab. Cells isolated from ventral spinal cord failed to migrate from the site of injection whereas dorsal cord cells did migrate. This difference may result from the fact that more cells in the dorsal cord are undifferentiated and still dividing at st 26. The final location of the dorsal cells was dependent on the developmental stage of the host. When injected into st 16-19 hosts, dorsal spinal cells migrated into sympathetic ganglia and peripheral nerves, but in st 22-24 embryos these cells migrated to DRG, peripheral nerve and skin. The migration of spinal cells to different locations at different stages parallels the sequential developmental changes of crest phenotypes.

These results show that at least some undifferentiated neuroepithelial cells in the dorsal spinal cord retain the ability to develop a variety of neural crest-like phenotypes. Their differentiation as neural crest-like cells is dependent on the temporal environment they encounter during development. Supported by the NIH and the McKnight Foundation.


Previously, we have demonstrated that cardiac selective expression of NOF in a transgenic mouse produced sympathetic hyperinnervation and hypertrophy of an unknown cell population within the base of the heart (Hassahkani et al, Soc Neurosci Abst 18:1289, 1992). To further study this cell population, we have undertaken morphological, immunocytotoxic and physiological studies. Ectopic cells prepared from the base of hearts of 3-4 week old transgenic mice were dissociated and cultured in BIBS collagen, or 10% FCS. None of these cells showed spontaneous contractile activity and did not stain with an antibody against sarcomeric actin, ruling out myogenic phenotype. In primary culture, only 0.4-2% of the population was stained for GAP-43 and vimentin. Physiological studies revealed that they were not electrically excitable that they were poorly coupled. Unitary junctional conductance of these cells were 60 pS and junctional conductances were 0.4-2 nS. After 7 DIV, a second subpopulation of cells that adopt a fibro-like morphology were detected. These cells immunostained for glialFRA and peripherin. Overall, these results indicate that these ectopic cells are likely derived from neural crest. Experiments are being carried out to evaluate whether these cells arise by abnormal migration into the developing heart or abnormal mitogenic expansion of a normal cardiac neural crest-derived cell type.
614.15 TRANSIENTLY CATECHOLAMINERGIC CELLS IN THE BOWEL OF THE FETAL RAT EXPRESS mRNA FOR THE NMDAR1 RECEPTOR. M.S. Cumming*, G.A. Burns, C. Ullberns, and K.E. Stephenson, Department of VCAPP, Washington State University, Pullman, WA 99164.

Tyrosine hydroxylase-like immunoreactivity (TH-ll) has been shown to be a transient marker for precursors of enteric neurons (Baegte, Dev Biol 141:353 '90). In this study, we sought to ascertain whether neural crest cells, destined to innervate the fetal gut, express mRNA for the NMDAR1 subtype of the glutamate receptor. Serial sections of embryonic day 12 rat tissues were initially reacted with a polyclonal TH primary antisera (Peninsula Labs), using a standard avidin-biotin immunocytochemical protocol with DAB as the chromagen. The same sections were then hybridized with a 1.4 kb NMDAR1 riboprobe, which had been transcribed in the presence of 35S-UTP. The immuno-reacted/hybridized tissues were coated with radiosensitive emulsion and incubated for 5 weeks at -70'C. Clusters of silver grains were observed over cells exhibiting TH-ll in the primitive gut. The expression of NMDAR1 mRNA by TH-reactive cells suggests a possible developmental role for this receptor, such as neuronal migration.


The microphthalmia (mi) gene encodes a novel member of the developmentally important family of basic-helix-loop-helix-zipper transcription factors. Mice with mutations in this gene may be completely white, deaf and virtually blind. These abnormalities are due to deficiencies in neural crest-derived melanocytes that populate the skin and the stria vascularis of the ear, and deficiencies in the pigment epithelium (PE) of the retina that lead to microphthalmia.

To better define the pathogenetic mechanisms leading to these abnormalities, we now study the expression of this gene during development of normal and mi mutant embryos in situ hybridization. In wild type embryos, mi expression was first found at E8.5 in the PE and E11.5 in cells surrounding the otic vesicle (OV). TRP2, a marker for melanoblasts, was expressed almost as early as mi and apparently in the same cells. While mi expression started to decrease at E12.5 in the PE and E14.5 in the OV and was barely detectable at birth, TRP2 expression remained detectable even after birth both in the PE and in the stria vascularis. In embryos homozygous for a transgenic insertion at mi, expression of mi was weak in the PE and essentially non-detectable in the OV. TRP2, however, could be detected in the PE, but not in the OV, consistent with the observation that PE cells seem to remain intact, while neural crest-derived melanoblasts of the OV apparently do not. In embryos homozygous for a single cDNA deletion in mi that does not impair the gene's expression, both mi and TRP2 gave strong signals in the PE. However, in the OV, the expression of either gene remained low and became undetectable after E13.5, again suggesting absence of melanocytes. Thus, mi may play a role in melanoblast proliferation and differentiation but not the maintenance of the differentiated melanocyte phenotype.

615.1 DENDRITIC DIFFERENTIATION OF RETINAL GANGLION CELLS IN CHICK. R.L. Snow, D.J. Stelzner* and J.A. Robson, Dept. of Anatomy and Cell Biology, SUNY HSC, Syracuse, NY 13210.

Development of dendritic morphology of chick retinal ganglion cells was investigated using retrograde transport of Dil. Label was applied to the optic nerves of embryos fixed at E4 and E14. The embryos were stored in fixative for 6-8 weeks at 37°C. Retinal wholemounts were examined using epifluorescence and confocal microscopy. Selected retinas were photocovered and drawings of labeled cells were made using a computerized microscope.

At E6 many ganglion cells are still migrating. These cells are bipolar in shape and lack dendrites. They have axons in the optic nerve and trilling processes attached to the scleral margin. By E8 rapid dendritic growth is prevalent and trilling processes retrace. This early dendritic growth is characterized by the appearance of many short spiny projections from all sides of the soma. As the cells mature most of these spiny processes appear to retract. However, a few elongated and branchy processes giving the cells a mossy appearance. Later, many of these branches disappear. As dendrites continue to mature, distinct ganglion cell morphologies become evident. Gadolinium fields differentiate first, followed by several other distinct classes. [Supported by NIH grant EY03490]
615.3 NEUROBLAST MIGRATION IN THE DEVELOPING CHICK OPTIC TECTUM VIA PERIKARYAL TRANSLATION. J.A. Robson* and R.L. Snow, Dept. of Anatomy and Cell Biology, SUNY HSC, Syracuse, NY 13210.

Migration of neuroblasts in the optic tectum was studied using immunocytochemistry and retrograde transport of Dil. The results demonstrate that early neuroblasts do not migrate along radial glial processes. Instead, they retain connections with both the ventricular and pial surfaces while growing axons and translocating their nuclei. Immunocytochemical studies using an antibody specific for postmitotic neurons (TUJ1) reveal a cohort of bipolar cells spanning the neuroepithelium during the early period of cell migration (E4-E7). In older embryos, the immunocytochemical label is limited to postmitotic neurons with no tranning processes extending to the ventricular surface. Migrating neuroblasts were retrogradely labeled by applying Dil to the lateral margin of the tectum in embryos fixed between E4-E10. 6-8 weeks later the tissue was sectioned using a vibratome and examined using fluorescent and confocal microscopy. Many retrogradely labeled cells have a bipolar shape with leading and trailing processes contacting the pial and ventricular surfaces. Axons grow from the leading processes before somata reach their final locations and prior to retraction of trailing processes. Several sections were photocovered, mounted in Epon and serially sectioned at 2μm. Reconstructions of these cells show that labeling is not transcellular. These results support the hypothesis that many neuroblasts in thin tissues migrate by translocating their somata while retaining connections with the pial and/or ventricular surfaces.


During metamorphosis of the moth, the musculature of the abdomen is reorganized. While most of the larval muscles either die or are respecified during metamorphosis, several larval intersegmental muscles are maintained in a segment-specific manner; 0 in abdominal segments 1 and 2 (A1-A2) and A7-A8, in A3, and 5 in A4-A6. These muscles are used to carry out pupal-specific behaviors. In segments A4-A6, denervation triggers regeneration of two maintained muscles, the ventral internal medial muscle (VIM) and the ventral intercalated oblique muscle (VIO). Following denervation, both the VIO and the VIM were reduced to 33% of the normal size by the end of metamorphosis. Other maintained muscles are unaffected by denervation. We compared aspects of the induced degeneration in the denervated VIO with the normal degeneration in respecified muscles. Following denervation of the VIO, dying nuclei are observed between the second and the sixth day of pupal life (P2 through P4). In response present from P2 through P4. We used the thymidine analog 5-bromoodeoxyuridine (5-BrdU) to label nuclei undergoing DNA replication. No nuclei label in the innervated VIO, while some nuclei in the denervated VIO label beginning on P4. 5-BrdU labeling also begins on P4 in respecified muscles. The external fibers in the denervated VIO degenerate, while the internal fibers remain intact. It is the degeneration of the external fibers which leads to the reduced size of the denervated muscles. Unlike respecified muscles, no new adult fibers develop.

615.7 CHICK EMBRYO PURKINJE CELL DENDRITES ARE REDUCED IN SIZE FOLLOWING CHRONIC TREATMENT WITH A NMDA RECEPTOR ANTAGONIST, NPC 12626. B. White* and M.W. Vogel, MD Psych. Res. Center, UMBAR, Baltimore, MD 21228.

The development of Purkinje cell dendrites in the chick cerebellum interacts with afferents. The role of neuronal activity in this interaction is investigated by chronically treating chick embryos with a NMDA receptor antagonist. The NPC 12626 (Glytux Pharmaceuticals) was chosen as the target for pharmacological blockade because of its importance in synaptic stabilization, and because granule and Purkinje cells are more sensitive to NMDA early in development, suggesting that NMDA receptor activation is important for cerebellar development. Chick embryos were given daily injections from E14 to E17 of the competitive NMDA receptor antagonist, NPC 12626 (Glytux Pharmaceuticals). The dosage was increased daily, with a low dose group starting at 20 mg/kg (end 100 mg/kg) and a high dose group starting at 50 mg/kg (end 200 mg/kg). Drug-treated (n=52) and control (n=19) embryos were killed on E18 (HST 43 to 44) and their brains processed for Golgi-Cox staining. Purkinje cell dendritic arbors were measured using MacMeasur (S4 Purkinje cell/cerebellum).

The NPC 12626 treatment significantly reduced embryo motility after the first injections, but motility increased to control levels during the following days. The morphology of Purkinje cell dendrites in drug-treated embryos appeared to be within the range of normal variation. Morphometric analysis showed, however, that there was a 20% reduction in the average size of Purkinje cell dendritic arbors. The results suggest that NMDA receptor activation is involved in Purkinje cell dendritic growth. Research support provided by NIH grant NS29727.


During formation of the leech excretory system, the ecotodermal bladder contacts the mesodermal nephridia. Sensory and neurosecretory innervation of each excretory system occurs by a single 5-HT neuron (NNC). The NNCs contain, and presumably release, FMRFamide at (1) the urine-forming cells, (2) the ureteric sphincter muscles and (3) central neurons (Wenning et al., J Exp. Biol. 1982). Removal of the prospective bladder cells between embryonic days 8 and 10 (E8-10) causes the nephridium to degenerate. Until E16, however, its differentiation into two types of urine-forming cells is undisturbed, whether or not the NNC has been removed as well. As seen using immunofluorescence microscopy, the NNC contacts only differentiated parts of the excretory system (Wenning et al. Roux Arch Dev Biol 202, '83). At E16, normal nephridia contact their bladders and become functional, and the NNCs sprout extensively. The nephridia lacking bladders stop growing and degenerate. The NNC projections lose their immunoreactivity in the nephridium but not at the bladder. Degeneration of the nephridium is not due to a missing influence since the ecodermal urethra with its mesodermal musculature develop normally and persist. Thus, to form a functional excretory system, the nephridia must contact the bladder. Whether a special cell or subset of bladder cells (as described for Hebdobella in Mantlindale & Shanklin, Dev Biol 125, '88) is required is not yet known.

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615.6 DIFFERENTIAL EFFECTS OF CORTICOSTEROIDS ON NEURONAL AND GLIAL CELLS IN THE DENTATE GYRUS OF ADULT RAT HIPPOCAMPUS. B. Liao* and E.C. Armenta, Dept. of Biology, New York U., Wash. Sq, NY, NY 10003.

Corticosteroids can enter the brain and regulate cell proliferation and differentiation. The hippocampus, as a central component in the limbic system, is a major target of corticosteroids. Long-term (2 mo) adrenalectomy (LT-ADX) induces a loss of Nissl staining and 5-HT+IR in the hippocampal dentate gyrus. Short-term desametamethone (ST-DX) treatment of the LT-42D rats for 24-72 hr produces a rapid recovery of 5-HT+IR, followed by a reappearance of Nissl staining. In the present study, we further explored the neuronal and glial response to the change of corticosteroid level. Female Sprague-Dawley rats weighing 150 g were adrenalectomized and given saline to drink. Sham operated rats were given water drink. After 2 months, some of the ADX rats were given DEX (10 μl) in saline drink for 72 hr. Bromo-deoxyuridine (BrdU) (50 mg/kg BW, i.p.) was injected into all three groups of rats at 24 and 48 hr later after the start of DEX to one group of ADX rats. LT-ADX produced a loss of cabaline immunoreactive (CAβ-IR) granule cells, which included a decrease in cell number, a swelling and pale-stained cytoplasm, a decrease in the density of dendrites, and a narrower dentate molecular layer. Double immunostaining for histone showed that small nuclei without cell body were sparsely distributed within the granule cell layer. ST-DX to LT-42D rats reversed partly all of above consequences. No massive neurogenesis after LT-42D or ST-DX were observed. After LT-42D, numerous BrdU-IR cells emerged in the dentate area, which corresponded to BrdU-IR area. ST-DX did not increase the BrdU-IR cell number, but promoted the elongation of the Vm-IR cell processes.

The evidence suggests a neuronal de differentiation and astroglial proliferation after the loss of corticosteroid support (Supported by NIA P01 AG10280 to ECA).


To study the expression of the Purkinje cell layer in the frog cerebellum during metamorphosis, tadpoles of Rana pipiens were immersed in a thyroxine solution (1 part per 100 million) to accelerate the metamorphic process, and batches of tadpoles along with control animals were anesthetized and killed at 2 day intervals up to 30 days of thyroxine exposure. The brains were fixed in Bouin's fluid for 3 hours, rinsed in buffer and embedded in paraffin. Immunocytochemistry was done on Bouin's fluid fixed, paraffin embedded sections using anti-calbindin D-28K, rabbit antisera (Swant, Switzerland), biotin conjugated secondary antibody, and visualized with a peroxidase chromogen.

Purkinje cells were immunoreactive at all stages of development, although the pattern of distribution was not uniform. We also noted a distinct gap in the Purkinje cell layer at a site where a cone of cells forms in the external granular layer (egl). Since the gap is present prior to the formation of the egl, the cone may not be responsible for forming the gap, rather, the cone may form at the site where a gap already exists. In addition to Purkinje cells, a population of small cells in the curricular lobes and the dorsal part of the corpus cerebelli were also immunoreactive. Because these small cells resemble the cells involved in postmetamorphic differentiation, our earlier contention that these may represent immature Purkinje cells may not be totally accurate. While some small cells which are immunoreactive during metamorphosis may be immature Purkinje cells, others appear to be cells of the extracerebellar granular band. Immunoreactive commissural fibers in the dorsal of the molecular layer of the cerebellum may be processes of these cells. Supported by NSAA Grant AA75377.
615.9 MORPHODIFFERENTIATION OF SUPRAEPENDYMAL CELLS: THEIR INTERACTIONS AND DEVELOPMENT OF THE SUBCOMMISSURAL ORGAN

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The subcommissural organ (SCO) differentiates between days 10 and 16 gestational development in the rat, it is probable that supraependymal cells (SEC) participate in its differentiation. In this model the SCO’s susceptibility increases to diverse factors which could produce display and induced hydrocephalus. After learning, the SEC’s ontogeny with a scanning versus neocortical involvement in the neural type SEC (Kölliker I) and fagocytosis (Kölliker II) during their apposition in the ventricular lumen, migration: now we are observing their interactions and superficial changes (probably laminin, fibronectin and adhesion) using SEM. The fagocytic SEC appear with vesicles and filaments in the ventricular surface, in adjacent zones to the proliferative neuroependym (PNE), and in the infundibular region. In the dorsal ventricular zones there appears to be less vesiculation; part of their prolagnations in the (KL) seem to be flattened other cells present an irregular discoidal shape in ventricular surface. Fibriillary elements interconnect with the PNE near the blood vessels. Interpendymal cell unions with a central clila may be prominent or in a groove like form depending on the region analyzed. SEC and ependymocytes fuse together in some cases in others, the presence of pores suggest other types of SEC communication between intra and extraventricular spaces. We will continue with immunocytochemistry identifying this interaction. Acknowledgement for support of this work is provided by the V. DeP. Anatomia and Div. Investigacion y Posgrado Fac. de Medicina UNAM.


The subependymal layer (SL) is derived from the fetal subventricular zone and in the adult rat retains developmentally regulated adhesiveness as well as immature dividing cells. This study examined the hypothesis that mitosis, growth factors, cell phenotypes, and proliferation all contribute to the subependymal layer. In particular, the subependymal layer was analyzed after the removal of the SL, at the time of injury or reattachment at the adult rat. The number of Nissl-stained cells in the SL increased 2-fold after unilateral cortical lesions. However, the number of BrdU-labeled cells in the SL of the adult rat decreased and very few BrdU-labeled cells were observed outside of the SL suggesting that mitosis and migration remained constant after resection. Therefore the increased cell number in the SL may be due to decreases in the rate of cell death after cortical injury. The growth promoting factors fibroblast growth factor (bFGF) and epidermal growth factor (EGF) were also examined after resection. Whereas a robust decrease in the expression of bFGF was observed in the SL following cortical lesions, EGF was unaffected in the subependymal layer before and after resection. Cells isolated from adult subependymal layer can express neuronal or glial phenotypes in vitro. In vivo, however, cells in the subependymal layer did not express mature neuronal (synaptophysin, neuron-specific enolase, MAP-2) or astrocytic (GFAP) phenotypes before or after lesion. Vimentin—immature astrocyte protein—positive radial glia in the medial striatum also expressed GFAP after lesion. Immunoactivity to polysialylated neural cell adhesion molecule (PSA-NCAM) increased 3-fold in the SL and medial striatum after cortical lesion. In contrast, immunoactivity to tenascin decreased transiently in the SL and medial striatum after cortical lesions. This study demonstrates that in response to cortical lesion, the adult SL is a highly plastic area with regards to cell number and adhesion molecules. Supported by PH Grant NS29230.


The present study emphasize to characterize the morphological and neurological differentiation of mesencephalic dopaminergic neurons from human embryos, derived from elective first trimester gestations. Embryonic brain tissue was taken for analyses of tyrosine hydroxylase (TH) by immunohistochemistry and Western blot, and for analyses of endogenous dopamine (DA) content using HPLC-ECD. TH was first detected immunohistochemically at 4.5 weeks of gestational age. In parasagittal sections of embryonic brainstem at this developmental stage, a small, distinct population of rounded, densely packed, TH-immunoreactive perikaryon was seen in the mesencephalic tractus. These cells were located in the middle of the margin of the ventral base plate, parallel to the ventricular zone. During the sixth to tenth gestational week, the number of TH-positive cells increased exponentially as a function of the TH-immunoreactivity. TH-positive cells migrated ventrally and somewhat rostrally, away from the ventricular marginal layer. During this period, short primary processes developed into long axonal trajectories giving rise to the nearest neocortex marginalis and the periventricular zone. At the tenth gestational week, varicose fibers were detected in most areas of the striatal anlage. To confirm the identity of TH in the embryonic tissue, as recognized by the antisera used for immunohistochemistry (Pel-Freez, USA), mesencephalic tissue of 5-10 weeks of gestation was analyzed by Western blot technique. A single band with the molecular weight of 60 kDa was detected already at 5 weeks of age. The amount of TH protein increased approximately ten-fold during these developmental stages. Mesencephalic tissue and forebrain/basal ganglia ganglia was taken for analyses of dopamine (DA) content using HPLC-ECD. DA could be detected at 5.5 weeks of gestational age in both areas and was found to increase exponentially from 7.7-5 weeks of age to reach 4.5 - 0.55 mg DA/mesencephalon and 50-75 mg DA/caudate nucleus at the end of the first trimester.


We have identified two distinct chondroitin sulfate proteoglycan (CSPG) spots in chick brain which can be differentially controlled by their immunoreactivity with S103L mAb, which recognizes an epitope in the aggrecan core protein, and HNK-1 (Kruengr et al., 1997). The expression of this CSPG is regulated both temporally and spatially throughout neuronal ontogenesis at high levels and constitutively in neuronal cultures as compared to glial cultures. In addition, astrocytes produce an abundance of CSPG with a larger core protein (>500 kDa), probably related to versican. Our lab has recently identified a mutation in the aggrecan gene in the nanomelic chick. The mutation introduces a stop codon in the translated sequence, and as a consequence a truncated core protein is synthesized which is not glycosylated or secreted in chondrocytes (Li et al., 1993). No expression of the complete S103L CSPG was found in nanomelic brain but the expression of the other brain CSPGs was not affected. Synthesis and processing of the S103L CSPG core protein as well as the functional consequences were studied in nanomelic neuronal culture. The regular order of aggregates was dramatically interrupted, with neurons often traveling in parallel. These data further support our hypothesis that the S103L CSPG functions to halt neuronal migration and together with sequence data confirm that the S103L CSPG and the core protein are products of the same gene. (HD 09402)

615.13 CLINICAL CORRELATES OF UNRATIONAL COLUMNAR ORGANIZATION OF CORTICAL AREAS IN CHILDREN WITH CHOROID PECHE FISTULA. E. Kogut*, G. A. Barver, H. Deuchow, J. J. Hoppe, J. J. Hopper, J. T. Barrett, A. Al-Saif, J. Alvarez. Mina Children's Hospital, Department of Neurosurgery, Kula, FI 8355.

Developmental pathway is a well recognized cause of chronic epilepsy and mental retardation, yet there are few studies linking specific histopathologic abnormality with potential outcome. We report the clinical findings of 18 children (12 females, 6 males aged 3 months to 15 years (mean 7.1, SD 4.7) with abnormalities of cortical neurones, migration, and organization. All had undergone focal cortical resection (hemispherectomy or multilobar 9, frontal 5, temporal 4) for medically resistant partial seizures. Tissue analysis revealed lamellar pattern of cortical cytoarchitecture, lamellar arrangement of neuronal layers, and lamellar alignment of cerebral cortical organization than the usual horizontal lamination. Oligo-axonal white matter (W-M) while white matter gliosis (W-M), ischemicentially abnormal (I-M) were recognized occasionally blemished or multinucleated cells (M-M), and variable cortical width (W-M) were also identified. Mean age of seizure onset was 1.8 years (1 month to 7 years). Mean seizure duration (M-M) were 2 years (3 months to 13 years, SD 3.4). Fourteen children experienced daily secondary generalized motor convulsions. Eleven children were developmentally delayed and 5 functionally impaired. These findings indicate that abnormal columnar cortical organization occurs in association with other abnormalities of neurones, gliosis, and neuronal organization. This aneibe, cortical cytoarchitecture correlates with early onset epilepsy, widespread epileptomorphicity, and a high frequency of developmental delay.

615.14 DEVELOPMENT OF THE NEOCORTEX OF THE MOUSE USING BrdU IMMUNOCYTOCHEMISTRY. Dr. P. Humphreys, Dr. W. Hendelmann*, and L. Long. Dept. of Pediatrics (Neurology) and Anatomy & Neurobiology, University of Ottawa, Faculty of Medicine, Ottawa, ON, Canada, K1H 8M5.

Previous studies attempting to define the time of birth of neurons destined for layers II-VI of the mouse neocortex have given conflicting results, possibly due to differences in definition of embryonic day numbers, relatively long time periods (up to 12 hours) during which cells is permits to occur, and cell-strain variation. We have timed the generation of neocortical neurons in the Paris R-3 mouse. Animals were mated over periods varying from 1.5 to 5.5 hr (average 2.5 hrs); the day of conception was considered embryonic day 0.5. Pregnant mothers were given an intraperinatal injection of 5-bromo-deoxyuridine (BrdU, 100 mg/kg) at one of six precise times: E14.0, E14.5, E15.0, E15.5, E16.0, and E16.5. Ovulations were determined by visual inspection. Pregnant mothers were sacrificed on any of the following periods: 1 P1), P9 and P22. Brains were fixed in 10% ethanol, coronally sectioned at the anterior hippocampal level, and cut into 50-80 um sections. Brains were processed with the immunoperoxidase method (deFazio et al., 1987). BrdU-labeled nuclei in the dorsolateral cortical plate (P1) or neocortex (P9, P22) were counted using an image analyzer. Analysis of data produced two general findings that neurons born at 6 injection times were concentrated predominantly in specific layers as follows: E13.0-IV-VI; E14.5-IV-V; E15.0-III-V; E15.0-III-V; E15.0-II-I; E15.0-I-II; E15.0-III. (Supported by grants from the Children's Hospital of Eastern Ontario Research Foundation and the Bickell Foundation.)
616.1

MICRÓGLIAL REACTIVITY TO METHYL-D-ASPARTATE (NMDA) INDUCED EXCITOTOXITY IN THE EARLY POSTNATAL BRAIN. L. Azevin, B. Gonzalez, B. Caamaño and A. Cuesta. Dept. of Cell Biology, Neuroanatomy and Anatomy, University Chicago, Maywood, IL 60153; and Dept. of Cell Biology and Physiology, Autonomous University of Barcelona, Barcelona, Spain.

Injections of NMDA into the early postnatal brain causes severe and rapid neuroglial loss due to the excitotoxicity of the developing brain to this excitotoxicity. This excitotoxic process has been implicated in the neuronal damage occurring after hyperactive stimulus. In the present study the time course of microglial reaction was examined in cortical areas receiving NMDA injections as well as in cortical projection areas such as the parietal cortex. Under ether anesthesia, 6-day-old rats received an injection of 500 nmol NMDA (0.26 mg/ml) into the right sensorimotor cortex. At various times ranging from 10 hours to 2 days post lesion, microglial reactivity was studied using tissue immunohistochemistry.

Microglial reaction was seen as early as 10 hours after NMDA injections. Reactive cells in the neocortex, the hippocampus, and the rostral thalamus showed an asthmatic and pseudopod-like morphology. In the cortex, the microglia responded in association with autophagically degenerating cortical elements coursing through the pons. This reaction was already seen at 10 hours post injection, it peaked at day 1 and was nearly absent by day 7.

In summary, after NMDA injection in the early postnatal brain microglial reactivity was restricted to areas showing apparent neuronal or astrocyte degeneration. This reaction was characteristic of rapid onset of severe injury.

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616.3

GLIAL ACTIVITY IN WILSON'S DISEASE. S.C. Page, A.N. Kaletha, A. Chlouptkova, G. Raychov and G.W. Kreutzberg. Max Planck Institute for Psychiatry, Am Klopferspitz 18a, 8133 Martinsried, Germany, and Institute of Psychiatry and Neurology, 19 Sobelka Str, 02-957 Warsaw, Poland.

Wilson's Disease (WD) is an autonomic neurodegenerative disorder characterised by the toxic accumulation of copper in a number of organs, particularly the liver and brain. The disease affects approximately 1 in 30,000 live births. Recently the gene for WD has been mapped to chromosome 13q14. It has been suggested that the gene product, the copper transporting lysosomal protein (COP) is expressed in the liver. However, little is currently known of the mechanisms by which neuronal damage occurs in the brain tissue, notably in the basal ganglia, although histological techniques have shown that reactive glia is a feature of the disease.

This study uses post-mortem tissue from 3 patients exhibiting neurophysiological symptoms and from 9 controls. Cryosectioning and immunocytochemical studies in WD brain tissue, screening with a variety of antibodies was performed. GFAP staining was carried out on both frozen and formalin-fixed free-floating sections as a marker for astrocytic activity. Microglial cells were visualised using either EMRIA or specific HLA-DR for frozen and fixed-free floating sections respectively. An antibody against the MHC-II (HLA-DR) antigen was also employed.

GFAP staining revealed a large number of reactive astrocytes in the basal ganglia tissue, notably the putamen, in addition to the thalamus and cortex. HLA-DR immunoactivity was localised to glial cells, shown to be GFAP-positive and therefore astrocytic, in double-labeling experiments utilizing DAB as the initial chromogen and HRP as the second. HLA-DR immunoreactivity was present with high incidence in many areas of the brain, including the basal ganglia, cortex, internal capsule, and the cortex. Within these tissues, HLA-DR immunoreactivity appears to be primarily, although not exclusively, located in the white matter.

To further characterise the role of reactive glial cells in neurodegeneration in WD, current studies are examining substances that may be secreted by such cells and which subsequently lead to tissue degeneration.

616.5

TOPICAL GLUCOCORTICOID MODULATES RESPONSE TO CEREBRAL CORTICAL STAB WOUNDS. M.S. Lu, S. David Centre for Research in Neuroscience, Montreal General Hospital Research Institute, Montreal, Quebec, H3G 1A4

Penetrating injury to the CNS results in the formation of an interface (glia limits and fibrous scar) across which axons fail to regenerate. Its maturation is associated with the deposition of a basal lamina. We have examined the effect of topical steroids on the formation of this interface. Stab wounds (2 mm long and 1.5 mm deep) were made in the parietal cortex of adult rats. Immediately after lesioning animals received either 1) a topical application of halocortisone 0.1% (intact), 2) placebo treatment or 3) no treatment. Animals were perfused 3 weeks later. Cryostat sections were labelled with anti-GFP and anti-laminin antibodies and visualised by immunofluorescence. The length of laminin immunoreactivity along the lesion interface, and the number of reactive astrocytes 300 μm lateral to the lesion were quantified. Laminin deposition at the interface was diminished in the treated animals by 24% compared to controls. The place treatment effect was negligible at 2% astrocyte reactivity was decreased in both the steroid and placebo groups by 36% and 26% respectively. This reduction in astrocyte reactivity may be part be from the barrier properties of the cisterns, preventing migration of foreign material into the lesion. These results suggest that steroids could alter the formation of the interface and may have implications for axon growth across CNS lesions.

616.6

GLIAL RESPONSE IN THE RAT SEPTAL COMPLEX FOLLOWING BILATERAL LIMBIC FIBRILLATION TRANSECTION. E. Heilbeck, M. Fronicher, and T. Naumann. Institute of Anatomy, University of Freiburg, P.O. Box 111, Germany.

Lesion of the limbic-fornix leads to degenerative changes in the septal complex as well as in the hippocampal formation. In particular, many parts of the neuronal circuit take place in the septal complex, whereas the hippocampus is in the septum or the hippocampal formation. Thus, the septal complex is a very important area for the understanding of the pathophysiology of psychiatric diseases. The main target of this study was to examine the cellular response to a lesion of the septal complex using immunohistochemistry.

GLial reaction was studied in adult Sprague-Dawley rats, r, 5 weeks and 6 months following bilateral limbic-fornix transection (BF). Immunohistochemistry was carried out on vibratome sections from perfusion-fixed brains. In some animals MS neurons were pretreated by intraperitoneal injections prior to axotomy in order to document both the fate of septohippocampal neurons and glial phagocytosis ultrastructurally.

At all postlesional stages only few microglial cells were detectable immunocytochemically by using a GF. Thus, there is only limited neuronal death and glial phagocytosis is mainly limited to the lesioned area (MS). In contrast, a strong increase in GFAP and GST-β-H2 staining in the LS and hippocampus was observed already 1 week post lesion. Moreover, increase in glial staining was not restricted to areas affected by BF. The intensity of all glial markers returned to that of controls at about 6 weeks post lesion. Thus, glial reaction after BF is a wavy curve, Naumann et al. (1992) J. Comp. Neurol. 325:219-242.

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616.7
GLIA: IMMUNOREACTIVITY IN TRIGEMINAL GANGLION SATELLITE CELLS AFTER TOOTH INJURY IN RATS. J.L. Stephenson and M.R. Byers* Dept of Anesthesiology, University of Washington, Seattle, Washington, 98195 USA
An increase in glial fibrillary acidic protein (GFAP) immunoreactivity (IR) is often seen in astrocytes of the central nervous system in response to a wide variety of injuries. We have investigated injury induced GFAP-IR in ganglion satellite cells, in response to graded tooth injuries. We compared GFAP-IR of trigeminal ganglion (TG) after maximal mandibular molar injury at 1,3, and 7 days after the injury, and for different degrees of injury. Fixed ganglia (4% formaldehyde) were cut in the horizontal plane at 50 microns, and reacted with a 1:1 series of rabbit antigens encircled by reactive satellite cells. The number of neurons with satellite cell reaction in each experimental category was compared to the uninjured control TG, as well as to zones within each section that did not correspond to the site of injury. We found significant increases in the total number of GFAP-IR satellite cell profiles surrounding tooth related neuronal cell bodies in the 7 day group (p=0.016). The GFAP-IR at 3 days was dramatically increased, but with a larger variance in the total number of reactive cells than at seven days. The site of GFAP reaction in the TG, shifted depending on the injury site (max or mand). Satellite cell GFAP-IR corresponded to the zone labeled by retrograde labeling of neuronal cell bodies by Dil from the molars. EM confirmed the identity of the GFAP-IR cells to be satellite cells. We conclude that a GFAP-IR satellite cell reaction is induced in trigeminal ganglia by molar injury in a specific manner at 3-7 days. Supported by NIH DE05159.

616.8
FGF RECEPTOR EXPRESSION FOLLOWING TRAUMATIC BRAIN INJURY IN RATS. J. F. Reilly and V. K. Momoh* Department of Cell Biology & Human Anatomy, School of Medicine, University of California, Davis, CA 95616
Basic fibroblast growth factor (bFGF) is thought to play a role in astrogliosis following traumatic injury to the CNS. In the present study, trauma-induced changes in the spatial and temporal expression of FGF receptor 1 (FGFR-1, fgr) were examined. A stereotaxic lesion through the hippocampus was placed in adult, male Fisher 344 rats. After survival times ranging from 1 to 30 days, brains were removed and processed for immunohistochemistry using a monoclonal antibody to FGFR-1. Quantitative image analysis was carried out to evaluate the magnitude of the changes. Cortical expression of FGFR-1 was increased adjacent to the wound cavity by day 2 post-lesion. Levels peaked at day 4 and decreased to control levels by day 10, except for reactive astrocytes immediately adjacent to the wound cavity. In the hippocampus, FGFR-1 immunoreactivity was increased on day 4, peaked at day 7, and remained elevated beyond day 10. By day 30, staining had returned to control levels except for reactive astrocytes immediately adjacent to the wound cavity. Double immunohistochemistry for FGFR-1 and GFAP demonstrated that astrocytes are expressing FGFR-1. bFGF colocalized with FGFR-1, suggesting that FGFR-1-expressing astrocytes are also expressing bFGF. These data demonstrate a time course for astrocyte expression of FGFR-1 which precedes and parallels the established time course for astrocyte hypertrophy. This suggests that endogenous bFGF may act directly on astrocytes to induce astrogliosis.

616.9
TYROSINE PHOSPHORYLATION IN RAT SPINAL CORD AFTER SCATIC NERVE TRANSSECTION. W.A. Bickler1, J.O. Velatichoff,2 C.A. Ota2, A. Renton2 and R.J. Weintraub.3 Dept. of Physiology, and 2Cell Biology & Anatomy, UNC, Chapel Hill, NC 27599, and 3Dept. of Anatomy & Cell Biology, University of Virginia, Charlottesville, VA 22908
Nerve injury produces central changes reflecting both degeneration and regeneration of nerve fibers. Release of a growth factor, likely to act via receptor tyrosine kinases, may be an important signal associated with peripheral injury. Immunocytochemistry using antibodies for phosphotyrosine was employed to identify changes in tyrosine phosphorylation in the rat spinal cord consequent to sciatic nerve injury. Increased immunostaining in the spinal gray matter, dorsal columns and gracile nucleus on the side of the lesion became evident after three days and was more pronounced with longer survival times up to three weeks (the longest survival tested). This increase was most prominent in the fourth lumbar segment (the focus of termination of sciatic nerve afferents). Immunostaining was in astroglial cells and their processes in the dorsal horn; stained microglia was also seen. Immunopositivity also increased in glial cells surrounding motoneurons at the same levels. These results in combination with other work from our laboratory suggest that some factor released by primary afferent fibers over a sustained period results in sustained kinase activation, either directly or via a cascade involving sustained synthesis and release of other factors. This kinase activation is likely to play an important role in the full development of the glial response to nerve damage.

616.10
EXPRESSION OF A NOVEL NON-ANGIOTENSIN II [125I]CGP 42112 BINDING SITE IN HEALING WOUNDS OF THE RAT BRAIN. M. Viswanathan*, A.M. de Oliveira, F.M.A. Correa, and J.M. Savidra. Sec. on Pharmacology, LCS, Bldg. 10 Room 2D-45, National Institute of Mental Health, NIH, Bethesda, MD 20892
Angiotensin II (Ang II) receptor subtypes AT1 and AT2 seem to be involved in growth and repair processes during wound healing. Our studies using the AT1 ligand [125I]CGP 42112 unexpectedly revealed, in healing brain wounds of adult rats, a binding site that is recognized by CGP 42112 and not by angiotensin II. Using quantitative autoradiography, we localized and characterized this site. [125I]CGP 42112 binding was restricted to the wound edge and the immediate periphery. Immunocytochemical localization using ED-1 monoclonal antibody raised against rat macrophages showed good correlation between the distribution of ED-1+ cells and the binding which was saturable, reversible, and stable. Saturation studies and Scatchard analysis of the data revealed a single class of binding sites with a Kd 3.8X10^-1 M and binding capacity (Bmax) of 109 fmol/mg protein. The time course of injury-induced expression of the binding in the brain lesion revealed highest levels at 3 days after injury which became undetectable after 10 days. Our results suggest that activated microglia surrounding the penetrating wound express the novel binding site and that this site may have a role in mechanisms of tissue repair in the brain.

616.11
Brain injury results in activation of glial cells, including both astrocytes and microglia, and an increase in the expression of several endogenous pro-inflammatory cytokines such as IL-15 and TNF-α. Increased expression of these cytokines has also been observed in the brains of individuals with Alzheimer's and Parkinson's disease. Previous studies indicate that astrocytes make both prostaglandins (PG) and nitric oxide (NO) in response to IL-15. To determine whether cytokines influence the expression of genes responsible for the generation of PG and NO, we performed northern blot analyses of RNA from purified primary cultures of astrocytes and microglia which had been treated with various factors. Our results indicate that PGHS-2, but not PGHS-1, mRNA is strongly induced in astrocytes exposed to recombinant IL-15 in a dose-dependent fashion. Induction is highest at 2 h, and PGHS-2 mRNA levels return to control values by 24 h. Macrophages/inducible NOS mRNA levels are also increased in astrocytes treated with IL-15, and peak at a later time point (4 to 8 h of exposure). TNF-α, LPS, and basic FGF also induce PGHS-2 in astrocytes, whereas demethylxanezone pretreatment diminishes the level of PGHS-2 induced in response to IL-15. Interestingly, PGHS-2 mRNA was not detected in cultured microglia treated with IL-15 or GM-CSF; however, PGHS-1 mRNA was present in these cells under all conditions tested. In summary, our results indicate that brain glial cells transiently increase their expression of synthetic enzymes for PG and NO in response to cytokines and suggest the elaboration of a cascade of mediators for cytokine action in the brain. [Supported by LEAD award AG00018 to P.D.C. and M.K.O.J.]

616.12
EFFECTS OF PLATINUM-RELATED CHEMOTHERAPEUTIC AGENTS SUCH AS CIPLATIN AND CARBOPLATIN ON NEUROSCIENCE ACTIVITIES OF NIH MOUSE SCHWANN CELL LINE. Y. Chagou* and R.R. Kim. Div. of Neurology, Dept. of Medicine, Univ. of British Columbia, Vancouver, BC, Canada, V6T 2B5.
Specific toxic effects of platinum-related chemotherapeutic agents such as ciplatin and carboplatin on neurons or Schwann cells have not yet been thoroughly evaluated. The NIH mouse Schwann cell line (Watabe et al., J. Neurochem Exp Neurol, 49:455, 1989) was exposed to ciplatin and carboplatin, and the survival rates were measured by MT assay. Blocking effects of NFG, ACTH, and e-MSI against platinum toxicity were also examined. Ciplatin displayed a marked cytotoxicity at concentrations higher than 1 μg/ml and carboplatin at concentrations greater than 2 μg/ml. DGF protected cell death from cytotoxic effects of ciplatin and carboplatin at the concentration of 50 μg/ml. These results suggest that ciplatin and carboplatin can induce Schwann cell damage due to platinum-induced neurotoxicity and that ciplatin is far more toxic to Schwann cells than carboplatin.
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616.13


Treatment with the anti-tumor drug cisplatin (CPl) leads to sensory neuropathy in patients, indicating that CPl affects the dorsal root ganglia (DRGs). In vitro CPl inhibition neurite outgrowth from DRGs, (2) decrease neurite length of DRG cells, and (3) reduce cell survival and neurite outgrowth to Schwann cells. As glial cells seem to be very sensitive to CPl treatment, we studied the role of these cells in neuroprotection. These studies were extended to other cell lines obtained from E15 rat DRGs and Schwann cells from P4 rat sciatic nerves. The sensory neurons were treated with 1 μg/ml CPl, which reduced neurite outgrowth with 37% (measured with fluorescence studies). After treatment of 5 μg/ml CPl with conditioned medium from glial cells or medium containing 15 ng/ml BNGF, Schwann cell CM and BNGF diminished the toxic effect on outgrowth with 60%, but satellite cell CM was not effective. These results suggest that Schwann cells and not satellite cells produce a soluble factor that protects against CPl-induced neurotoxicity. BNGF also had a potent protective effect in the peripheral nerve system. As Schwann cells are known to produce NGF, the Schwann cell derived protective factor may be NGF.

616.14

LEAD INDUCED DEVELOPMENTAL ALTERATIONS IN GLIAL GENE EXPRESSION. N. Zawia* and E. J. Bailey. Environmental Immunology & Neurobiology, Laboratory of Biochemical Risk Assessment, N.I.H.S., P.O. Box 12323, KIP, NC 27709.

Lead is a neurotoxicant that is known to produce behavioral, biochemical and structural abnormalities in brain development. We have previously shown that lead treatment leads to decreased expression of glial cells in situ of glial gene expression. The goal of this study is to examine whether these alterations in gene expression are related to regional timetables of development. To accomplish this, two brain regions with distinct prenatal (brainstem) and postnatal (cerebellum) schedules of development were chosen. Long Evans hooded rats were lesioned at low levels of lead in utero and sacrificed in the drinking water of the dam before parturition. Tissue was then obtained from the brainstem and brainstem on postnatal days (PN): 3, 6, 9, 12, 15, 20, 25, 30, 40, and 50. No changes in either brain structure and weight or animal body weight were observed in the pups following such exposure to lead. Total RNA was isolated and probed for myelin basic protein (MBP), glial fibrillary acidic protein (GFAP) and actin gene expression by Northern analysis. In the cerebellum, lead produced long term elevations in both MBP and GFAP gene expression that began on PN17.20 and were maintained into adulthood, expression levels remaining unchanged. No such shifts in the expression of these genes were observed in the brainstem. These results suggest that glial gene expression is more susceptible to the effects of lead in actively developing regions and that the state of gene expression may be permanently altered by lead.

616.15


The development of neuroanatomical projections requires a complex series of spatial and temporally regulated cell interactions. The thalamocortical pathway is established proportionally, but grows most extensively in target cortical regions postnatally. This delay may be dependent upon target maturity, as well as neuronal cells. Exposure to methylaminooxethanol (MAA) (5) kills mitotically active cells and when administered late postnatally, appears to alter thalamocortical fiber organization. We investigated the effect of MAA treatment (of MRA) when administered on either embryonic day 19, 20 or 21. Analysis of single (intraventricular) and dual (intracerebral injection of MAA) and fiber connectivites (90 labeling of thalamus) was performed at birth, postnatal day (P) 5, and P10. GFAP staining was more pronounced in reactive astrocyte-like cells in allotopic white matter and intracerebral capitol at P10. Moreover, I-E1 stained macrophages accumulated in white matter at the earliest time point examined and were still evident even at P10, almost 2 weeks after the single injection of MAA. The degree of nonneuronal cell response appeared to correlate with the disruption of the Dil-labeled thalamocortical fibers. In some brains, disrupted projections, anamalous growth cone and fiber varicosities were seen at birth. The results suggest that the MAA treatment produced an abnormal gliotic and photogenic response in the fetal brain, one which can have longlasting effects on cellular organization and developing fiber projections. Supported by NRSA fellowship 8809, NIGMS grant 81507 and NIA grant AG10560.

616.16

AXONAL DEGENERATION CAUSES A SLIGHT INCREASE IN ASTROCYTE PROLIFERATION IN IN VIVO. G. Gispen, G. Frisch, I.A. Greven, E. Urraca, and P.M. Weller. The Miami Project and Department of Neurological Surgery, University of Miami School of Medicine, Miami, FL.

Mechanisms inducing glia in the CNS after injury are not well understood. Astrocytes (AS) proliferate and gliosis have been implicated under similar insults 2. In this study, we analyzed axonal injury on astrocyte proliferation and evaluated at various times post-injury and related to axonal degeneration and regeneration. Since the transplantation of Schwann cells (SC) into gliotic areas of the injured CNS is of therapeutic interest, we also assessed whether factors released in injured N-A-S cultures affected SC proliferation on axons. Cultures of purified embryonic rat dorsal root ganglion neurons (N) were transplanted onto AS or SC monolayers. After the neurons extended axons on the AS or SC, a fast axon was cut 1.5 mm from the neuronal cell bodies. Non-injured cultures were used as controls. To assess proliferation, the cultures were exposed to H-thymidine, 1, 3 and 7 days post-injury (DPI) and processed for autoradiography. On 1 and 2 DPI, AS proliferation was slightly increased and SC proliferation was increased to approximately the same extent. Neurofilament staining distal to the lesion disappeared more rapidly in N-A-S than in SC cultures. Eight DPI, axonal regeneration was observed, but neither AS or SC proliferation changed. In two experiments conditioned media collected from injured and non-injured N-A-S cultures decreased neurite-induced SC proliferation when compared to uninjured N-S cultures, as assessed by autoradiography. However, no difference was observed between media obtained from injured and non-injured N-S cultures. Our data demonstrate that AS proliferation in gliosis may be caused partially by factors released after axonal injury. Supported by NIH grant ST82059, NMS grant 1R01 2210-2 and The Miami Project.

616.17


Glial cells in culture express several subtypes of functional adrenergic receptors. In order to determine which receptor types are expressed for glia in vivo we examined normal, crushed, and transected optic nerves of the rabbit and rat using quantitative adrenergic receptor autoradiography. We also obtained preliminary data regarding the expression of adrenergic receptors in normal and damaged human optic nerve. High levels of α1, α2, and β receptors were seen in all the adult species examined. The level of the 1 receptor in the rabbit and rat forebrain but only α1. α2 and β receptors were observed in the normal rat and rabbit optic nerve, and were seen in low to moderate densities. Normal, as well as damaged, human optic nerve also exhibited adrenergic receptor binding limited to the 1 and β species. After unilateral optic nerve crush or transection, only 1 adrenergic receptors were increased. This increase in 1 receptors was first significant at day 7 and 28 post-injury in the rabbit and rat optic nerve, respectively. The expression of 1 receptors in the transected optic nerve continued to increase with time so that by 90 days post-injury, the density of 1 receptors in both the rabbit and rat optic nerve was among the highest in any age of the forebrain. Taken together, these data suggest that in vivo, glia α2 adrenergic receptors may provide a therapeutic target for regulation of human astrocyte functions including astrocytosis, inflammation, and the hypertrophy and proliferation that occurs in response to neuronal injury. (Supported by the VA and NIH)

616.18

DENERATION-INDUCED CHANGES IN ASTROCYTIC GENE EXPRESSION ARE ALTERED IN AGING MICE. P.A. Timmerman. University of Virginia, Departments of Neurology and Neuroscience, Charlottesville, VA 22908.

This study is designed to evaluate if the time course of changes in astrocytic gene expression associated with Wallerian degeneration is altered by normal aging. Denervation was induced in the dentate gyrus of adult (60-65 days) and aging (1-2 years) C57BL6 mice by a unilateral lesion of the entorhinal cortex. In situ hybridization was carried out using a radiolabeled probe for glial fibrillary acidic protein (GFAP) was used to detect changes in levels of GFAP mRNA. In adult mice, there was a peak in GFAP mRNA levels at days 2 post-lesion (DPL). GFAP mRNA levels declined at 4 DPL but did not return to control levels until about 3 weeks post-injury. In adult mice, the peak in GFAP mRNA levels was reduced in size and was delayed until 4 DPL. GFAP mRNA levels returned to near control in lesioned aging mice by 8-10 DPL. These results indicate that up-regulation of gene expression in reactive astrocytes is compromised in the CNS of aged mice. Compromised astrocytic function as a consequence of normal aging could limit the ability of astrocytes to provide the metabolic and functional support neurons in the CNS need to cope with the degeneration that characterizes pathologic conditions such as Alzheimer’s disease. Supported by the Alzheimer’s and Related Diseases Research Award Fund.
16.1.7 CALCICTON GEN-RELATED PEPTIDE IMMUNOREACTIVITY IN YOUNG MUSCULOSERON AFTW NERVE INJURY. E. M. Blake and R. G. Berk. Dep. of Anatomy & Cell Biology, University of Maryland, Baltimore, MD 21201. Changes in calcium gene-related peptide immuno-reactivity (CGRP-IR) were examined in musculoserosa of young rats after nerve injury to determine whether during postnatal development, CGRP-IR typical of adult moto-neurons was elicited by axonal injury. Hypoglossal nerve crush or transaction was performed in rats at 10, 14 or 21 days postnatal (dpp). Rats were killed by aldehyde perfusion at 1, 3, 7, 14, 20 days postoperative (dpo). Results from the 21 dpo injury cases resembled those in the adult rat. Increased CGRP-IR continued after nerve crush until regeneration was complete. After transaction, a biphasic response of CGRP-IR consisted of an early increase (1-3 dpo), a decrease to basal levels (7 dpo) and a second elevation (14-20 dpo) that persisted until regeneration occurred. No consistent increase in CGRP-IR was seen after either injury at 10 dpo and regeneration was meager and nerve cell death was substantial. The same injuries in 14 dpo rats produced intermediate changes in CGRP-IR, regeneration and cell death compared to those obtained in the 10 and 21 dpo rats. The results strengthen the idea that persistent up-regulation or down-regulation of CGRP can be used to predict successful regeneration or apoptosis of injured motorneurons.

16.1.8 ANDROGEN RECEPTOR mRNA EXPRESSION IN ADULT HAMSTER FACIAL MOTOR NEURONS (HMFN). S.M. Dziegielewski*, R. J. Handa, K.J. Jones, Dept. of Cell Biology, Neurobiology, Anatomy, Loyola University of Chicago, Maywood, IL 60153. We have previously demonstrated that systemic administration of exogenous testosterone propionate (TP) to adult hamsters enhances the rate of facial nerve regeneration following injury in both genders, with the effects significantly greater in males as compared to females. Further studies also indicate that these effects of TP occur via a mechanism involving androgen receptors (AR), which are present in both male and female HMFN. In this study, we investigated the hypothesis that the differential effects of androgens on regeneration rate in male/female HMFN are related to gender specific differences in the levels and/or regulation of AR mRNA in these populations. Four groups of male hamsters were used: intact and gonadectomized males/females. In situ hybridization was performed using an 35S-labeled antisense AR cRNA probe encoding 350 NTs of the ligand binding region. Slides were processed for routine autoradiography and quantitative analysis was performed using a computerized image analysis system. Preliminary results indicate that AR mRNA levels are increased in male HMFN when compared to females. Gender specific regulation was also noted; gonadectomy decreases AR mRNA levels in males, but not in females. Studies in progress to evaluate the interactive effects of hormonal, injury states on AR mRNA expression in HMFN. Supported by NIH grant NS28238.

16.1.9 EXPRESSION OF MICROTUBULE-ASSOCIATED PROTEINS (MAPs) IN NORMAL AND REGROWING CAT TROCHELAR MOTONEURONS. A. A. Book*, I. Fischer, and E. H. Murphy. Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129. While previous studies have shown that the levels of actin, tubulin, and neurofilament proteins are altered in peripheral neurons following axotomy, much less is known about the role of MAPs in axonal regeneration. In this study, we examined the expression of a variety of MAPs, by immunocytochemical methods, in both normal and neurologically denervated trochlear motoneurons (TMNs) of the cat. The trochlear nerve of each animal (n = 4) was transected where it curved between the occipital bone and inferior confluence, and animals were allowed to survive 2 weeks. Three untreated cats served as controls. Brain sections were incubated with antibodies recognizing a high molecular weight form of tau, termed HMW tau, MAP-2, MAP-3, MAP-6, and a phosphoform of MAP-2 (pMAP-2). Terminals of pMAP-2, normal control TMNs, HMW tau and MAP-2 were observed in cell bodies and processes. In contrast, MAP-2 was present specifically in dendrites while pMAP-2 was found only in axons. Following nerve transaction, the levels of HMW tau appeared to decrease in axotomized TMNs, as compared to controls in the unaxotomized nucleus of origin or control animals. MAP-2 was increased in the axons of axotomized TMNs and was also observed in some cell bodies of axotomized TMNs. The levels of MAP-2 and MAP-6 in axotomized TMNs appeared similar to controls. These results suggest a potential role for MAPs in the regeneration of peripheral nerve since their expression was specifically regulated following axotomy. (Supported by NIH Grant NS 24707).
617.7 GALANIN EXPRESSION IN SYMPTOMATIC CANCER AFTER PARTIAL AXOTOMY. A. M. Shadack, D. K. McElroy, and R. E. Zigmond. Dept of Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

One of the ways neurons of the sympathetic nervous system display plasticity after axotomy is by alteration of expression of certain neuropeptides, such as vasoactive intestinal peptide (VIP), substance P (SP), and galanin (GAL). The increase in GAL-like immunoreactivity (IR) in the superior cervical ganglion (SCG) was significantly reduced, though remaining above control levels even at 14 days (Sun et al., 1993). In the middle and inferior cervical ganglia (MCGC), the increase in IR occurs by 2 days, plateaus through day 7, and then decreases further by 14 days. An increase returning to control levels by 60 days. The further increase in GAL-IR in the MCGC between days 7 and 14 may be related to the distance between the site of axonal transection and the ganglion.

With the use of a retrograde fluorescent marker, fast blue, we have shown that GAL-IR is mainly found in axotomized neurons of the MCGC. In order to determine whether changes in GAL mRNA are also related to axotomized neurons, we performed in situ hybridization for GAL. Using fast blue applied to the cut nerve trunk to identify axotomized neurons, we observed a high degree of coincidence between GAL mRNA- and fast blue-containing cells most of which appeared in the middle cervical ganglion region.

The functions of VIP, SP, and GAL after axotomy are yet unknown. Two possible sites of action for these peptides are within the ganglion and/or at the site of transection. We have found an increase in VIP and GAL, and perhaps a small increase in SP, in the proximal portion of the nerve trunk after transection and a further increase after ligation of that transected nerve. This suggests that VIP and GAL produced in the sympathetic neurons after axotomy are transported anterogradely within their axons.

617.9 THE CLONING AND CHARACTERIZATION OF A cDNA ASSOCIATED WITH NEURODEGENERATION AND SCAR TISSUE REGENERATION IN THE RAT. M. De Leenheer, R. L. Nahid, Y. Liu, A. A. Wielcher, E. M. Shooter. and M. A. Rudell. Dept. of Physiology and Pharmacology, Loma Linda University, CA; T. Dept. of Neurobiology, Stanford University, Stanford, CA; The Brandeis and Aesculapius Branch, NIDR, NIH, Bethesda, MD.

We report the cloning and characterization of DA11, a full length cDNA clone isolated from a transgenic rat dorsal root ganglion (DRG) cDNA library. The sciatic nerve of Sprague Dawley rats was crushed and allowed to regenerate. At three days after the crush the ipsilateral DRG were pooled together and the total RNA extracted. The RNA was purified through an oligo (dT) cellfree column, and 5g of RNA was used to construct a cDNA library (DA). The DA library was screened twice by differential hybridization (De Leenheer et al., J. Neurosci. Res. 29:437-448, 1991) and the cDNAs found to be elevated after sciatic nerve crush were analyzed. Prehybridization of the library library confirmed the isolation of a 0.6 kb DA11 cDNA clone whose expression was found to be induced after nerve crush. DNA sequencing and analysis of this clone revealed that the DA11 cDNA contained a single open reading frame that coded for a protein of a calculated molecular weight of about 15 kD. Northern blot analysis showed that the DA11 cDNA recognizes a single ~0.6 kb mRNA species that showed a three-fold induction in the DRG ipsilateral to the sciatic nerve crush, and increased to the contralateral DRG. The 0.6 kb DA11 mRNA was detected in total RNA extracted from the lung, heart, brain, sciatic nerve and spinal cord tissue, but the highest level was found in RNA extracted from rat DRG. The relative levels of DA11 mRNA did not change in the sciatic nerve during Wallerian degeneration. The DA11 mRNA was also higher in the cerebral cortex during the first two weeks after birth and it was significantly induced in P12 cell line cells after treatment with nerve growth factor. Our data support the hypothesis that the DA11 mRNA may play a role during sciatic nerve regeneration and cerebral cortex post-natal development.


The development of an animal model greatly affects outcome following CNS injury. For example, peripheral nerve transection in neonates elicits profound neurodegeneration in adult animals. We have previously reported that the expression of type I pro-collagen is significantly lower in adult animals, suggesting that these animals may have a mechanism for controlling the underlying molecular mechanisms governing recovery following peripheral nerve injury. The following study utilizes a transgenic approach to examine the effects of transcriptionally regulated, chemically altered, or deleted transgenes on the transcriptional cell lineages undergong degeneration, regeneration, plastic reorganization, and transient repair. This approach utilizes the c-fos-lacz or c-jun-lacz mouse lines, the left telibial and peroneal nerves were transected and a 2mm piece removed in either PO or adult subjects. Animals were allowed to recover for 2 hrs, 1, 2, 5, 14, or 28 days post-injury. Both fos and jun were transversely induced in spinal cord dorsal horn. In addition, fos and jun were upregulated in spinal cord dorsal horn. At 2 h post-injury, the gene expression of c-fos-lacz or c-jun-lacz in the spinal cord dorsal horn was significantly increased in both tissues. The level of c-fos-lacz or c-jun-lacz expression was significantly lower in adults than in neonates. These results suggest that jun is post-translationally regulated in adult and not in neonates. Luminogenic expression of the luciferase reporter was seen in the proximal stump of the injured nerve and was observed during transection into dorsal root fibers supplying the sensory neurons. These observations suggest that thymosin B10 plays an essential role in axonal growth during development, regeneration and in synaptogenesis.

617.12 INDUCTION OF THYMOSIN B10-LACZ TRANSGENE IN REGENERATING MOTOR NERVE AXONS IN THE PERIPHERAL NERVE INJURY. J. C. Chen, M. Butler, J. W. Worbarter* and J. L. Morgan. Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ.

In an attempt to identify molecular markers for regeneration, we found a progenitor cell marker used by thymosin B10-LacZ to reproducibly express in the developing and regenerating segmental nervous system. Its expression levels reach a maximum around the region of axonal outgrowth and decline sharply after birth. Biochemically, thymosin B10 has been shown to be a pro-thymosin protein. A previously developed model of thymosin B10 was cloned and used to generate transgenic mice that carry a thymosin B10-LacZ transgene. Th-LacZ is first detected atembryonic day 8.5 in the nervous system. During late neuroembryogenesis, its expression is widely distributed in the developing nervous system, including retina, cerebellum, and dorsal root ganglion neurones and ganglion. In the adult, high levels of thymosin B10-LacZ expression are restricted to neurones that have elevated synaptic remodeling. In addition to its synaptic expression in the neuronal cell body, thymosin B10-LacZ-fused protein is also detected in dendrites and axons.

The expression of thymosin B10-LacZ was monitored following peripheral nerve injury. Five days after facial nerve transection, an induction of thymosin B10-LacZ was observed in motor neurons in the ipsilateral facial nucleus. The expression remained elevated for several weeks during regeneration. Induction of thymosin B10-LacZ in the spinal motor neurons of L4 & 5 was also detected following sciatic nerve transection. Increased blue staining in axons in the proximal stump of the injured nerve was observed and extended into dorsal root fibers supplying the sensory neurons. These observations suggest that thymosin B10-LacZ plays an essential role in axonal growth during development, regeneration and in synaptic remodeling.

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617.13 DIFFERENTIAL DISPLAY PCR IDENTIFIES CHANGES IN GENE EXPRESSION FOLLOWING RAT FEMORAL NERVE LESION IN THE CORTICAL REGIONS OF THE MOLECRULAR BRAIN. J. A. Archibald, B. L. Robinson, and E. J. Madison. Depts. of 1.Med. and 2.Neurology, Duke University Medical Center, and Research Services, P.V. Hosp., Durham, NC and 3.Neurology, Duke University Medical Center (RDM). The 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, even when the distal nerve branches are completely isolated from their end organs (Bhushan, J. Neurosci. 13 (5): 2752-2768 1993). To identify factors present in the denervated pathways which may underlie preferential motor reinnervation, we are starting to analyze lesion induced changes in mRNA expression using differential display PCR. (Liang and Pardee, Science, 227, 897-911, 1992). Total RNA was extracted from terminal motor and sensory branches from control nerves or nerves which had been transected and prevented from regenerating for two weeks. Subsets of the expressed mRNAs were reverse transcribed with an anchored dT3 primer, and amplified with PCR using end labeled random 10-mers. PCR products were separated by PAGE. Most labeled bands representing expressed mRNAs were similar between lesioned and control motor or sensory branches. Several bands were present only in lesioned motor or lesioned sensory branches, representing potential mRNAs which are expressed only under denervated conditions. Differentially expressed bands will be sequenced to determine the utility of this approach in identifying mRNAs that are up- or down-regulated following nerve transection. NS24040-09 (RDM) and VA Merit Review (RDM).

617.14 BDNF LEVELS INCREASE IN THE TERMINAL MOTOR BRANCH FOLLOWING LESIONS OF THE PARENT FEMORAL NERVE: A COMPARATIVE STUDY IN M. MURRABY, M. P. Raposa, J. A. Archibald, and E. J. Madison. Depts. of 1.Neurology (Neurosurgery), 2.Neurobiology, Duke University Medical Center, and Research Services, P.V. Hosp., Durham, NC and 3.Med. (RDM). A 273 bp fragment of mature BDNF (nt 2573-2846) was amplified from rat muscle RNA and cloned into the pGCRi vector (InVitrogen). An unrelated Hae III digested fragment of pGCRi (-100nts) was ligated into the Sal I site within the BDNF fragment (at position 2287) which was verified by DNA sequencing and used to make sense riboprobe. Increasing concentrations of riboprobe were then used to reprobe reverse transcriptase reactions of total RNA harvested from lesioned and non-lesioned terminal motor branch of the rat femoral nerve. The amount of radioactive label present in each band was determined by exposure of the gel to a Phosphorimager. Total counts were then used to construct a standard curve relating concentration of spiked cRNA to target RNA. In previous studies using ribonuclease protection assays Funakoshi et al. (Cell Biol. 1993: 123: 455-465) levels of BDNF were barely detectable in normal sciatic nerve. In our competitive PCR assays BDNF is easily detectable in normal sciatic nerve or normal motor nerve but is present at approximately 10-fold lower concentrations compared to muscle. The amount of BDNF in two week lesioned nerve increases 10-30 fold in the motor branch of the femoral nerve (terminal branch to the quadriceps muscle) as compared to unlesioned nerve. Experiments are currently underway to determine BDNF levels in the other terminal branch of the femoral nerve which is pure sensory. Supported by NS24040-09 (RDM) and VA Merit Review (RDM).

617.15 CONSTITUTIVE HEAT-SHOCK-70 mRNA IS INDUCED IN RESPONSE TO FACIAL NERVE AXOTOMY. M. M. Raposa, E. J. Madison, C.A. Head, and G.N. Kreutzberg. Dept. of Neurobiology, Max-Planck-Institut fuer Psychiatrie, Martinsried, and 1.Anatomical Inst. 1, University of Freiburg, Freiburg. We have shown previously that constitutive heat-shock-protein 70 (hsp70) is increased in the regenerating facial nucleus. In this study, we extended these findings and examined if hsp70 mRNA would be induced in the facial nucleus following peripheral nerve lesion. The transection was done in Wistar rats at the level of the stylo-mastoid foramen and animals were sacrificed 6h, 12h, 24h or 3d later. Facial nuclei from operated and sham operated rats were extracted and prepared for Northern blot analysis using a cDNA probe complimentary to constitutive hsp70 mRNA. Autoradiograms revealed a single band (ca. 2.5 Kbp) which appeared to be maximally induced in 24h following axotomy, as compared to controls. Preliminary results at 3 days post-lesion indicate that relative levels declined but remain elevated over time. The time course of hsp70 mRNA induction is consistent to that previously reported for constitutive hsp70 protein, where maximal protein levels were observed at 3 days post-axotomy and were maintained elevated thereafter. These results indicate that peripheral nerve lesion induces hsp70 mRNA. Further, these data suggest for a role of hsp70 in the early phase and long-term maintenance of the regeneration program of motorneurons.

617.16 EXPRESSION OF NITRIC OXIDE SYNTHETASE (NOS) IN MOTOR NEURONS AFTER AXOTOMY. A. G. L. Smith, R. S. Treadway, R. A. Neary, R. M. Zuniga, G. P. Rudge, and J. B. McQuarle. Inst. 1.Biol. & 2.Anat., City Univ. of New York Med. Sch., NY, NY 10031 and 3.Bio-Chem. Inst. of Hong Kong, Hong Kong. NOS, an enzyme for the synthesis of nitric oxide, becomes detectable in anterior horn cells following facial nerve lesion, and in motor neurons of cranial nerves after nerve avulsion. Since anterior horn cells died after axotomy, and inhibition of NOS reduced neuron death, it was postulated that NOS expression signals the impending death of injured cells. However, extensive studies have been made only when cranial nerves were cut at young age, and few cranial nerve axons have been expressed after Aplysia age when compared with axotomy at adults. We hypothesise that expression of NOS in anterior horn cells is involved in neuronal response to injury. In this study, we examined the time course of NOS expression in cranial nerve motor neurons. 2 weeks after axotomy, adult, female rats received either unilateral axotomy or transection of one of the cranial nerves. Animals were killed at 1 and 3 days, 1, 2, 3 and 6 wks post-operation (PO). Thirty-micron thick coronal sections cut through the brain stem were processed for RADPH-diaphorase reaction for NOS. Changes of RADPH diaphorase reaction were observed 3 days PO in the two motor nuclei ipsilateral to axotomy. In the vagus nucleus, increases in the number of NOS positive neurons and intensity of labelling persisted 6 wks PO. In the hypoglossal nucleus, the number of NOS neurons reached maximally 3 days and 2 wks, and declined 4 and 6 wks after crush and transection, respectively. These data suggest that NOS was expressed in cranial motor neurons in a time course which could be correlated with axon regeneration, consistent with the hypothesis that expression of NOS is involved in neuronal response to injury, possibly regulated by axon regeneration. 617.17 LONG-TERM CHANGES IN HEMEOPHIL AFTER PERIPHERAL NERVE INJURY. J. P. Swerts, N. Madure, L. Cambonie, M. J. Guignaud, J. Smith* and P. Courbard. Centre de Biologie du Developpement, U.M.R. 9925 C.N.R.S. Univ. P. Sabatier, Toulouse. France. Analysis of molecular environments that allow nerve repair has revealed the presence of hemeprotein in cut rat peripheral nerves and the dramatic increase in the level of this glycoprotein after nerve transection (Swerts et al., J. Biol. Chem., 267, 10596-10600). Although it is a soluble protein, hemeprotein is colocalized in the nerve with extracellular matrix proteins. Moreover, hemeprotein synthesis has been detected within the nerve itself; thus this plasma protein can no longer be considered as a liver-specific product. In the present study, we have documented long term changes in hemeprotein levels in permanently degenerated (transected) and regenerating (crushed) sciatic nerves, using western-blotting and immunohistochemical techniques. In both transected and crushed nerves, there was a 6-fold increase in hemeprotein content at day 2 post-injury and a 12-fold increase at day 7 in the distal part of the nerves, compared with contralateral intact nerves. Beyond the first week, the fate of hemeprotein was very different in each type of lesioned nerves: in transected nerves the level of hemeprotein kept increasing, reaching over 20 times the basal level three months after injury, whereas in crushed nerves, hemeprotein level declined progressively in a proximo-distal direction, returning to basal values 2 months post-injury. These results suggest that hemeprotein could be regulated negatively, directly or indirectly, by growing axons and support the idea that hemeprotein could be involved in the process of Wallerian degeneration and/or in nerve repair.

617.18 EXPRESSION OF BETA-ACIDIN mRNA IN MOTORONOBUS AFTER AXOTOMY. K. N. Corrella, I. M. Lund, B. L. Ruff* and J. B. McQuarle. V. Med. Ctr. and Case Western Res. Univ., Cleveland, OH 44106, USA. The peripheral neuron responds to axotomy by switching from translation of proteins needed for normal function (e.g. neurotrophic factors) to proteins needed for axonal outgrowth (e.g. actin and tubulin). At 1, 4, or 14 days after several peripheral nerves (e.g. sciatic nerve) have been cut (with contralateral sham crush), the L4/5 spinal cord was excised and histsected. Northern blots of mRNA showed an increase in beta-acidin mRNA at one day on the crush side compared to the sham side. This level then returned to constitutive levels by 14 days. We have used in situ hybridization to further investigate the expression of beta-acidin mRNA in axotomy. Riboprobes were synthesized using SP6 and digoxigenin labelled nucleotides and detected immunohistochemically using an antibody to beta-acidin. This antibody was labelled with Fluroco-Ruby. Preliminary in situ data shows that mRNA was present at 6 days to a much lesser extent in the axotomized side. There is no increase in beta-acidin mRNA after axotomy correlate with known increases in beta-acidin synthesis and axonal transport. (Supported by grants to I.G.M. from the DVA.)
MOLeCULAR CORRELATES OF PERIPHERAL AXONAL REGENERATION

THURSDAY PM

617.19 ANOXYCINE OF MOTOR NEURONS ELUVIATES c-JUN AND JUN D LEVELS.
L.-W. Lui, L.-J. Campbell & I.-G. McQuarrie* VA Medical Center, and Case Western Reserve University, Cleveland, OH. Hypoxia-induced quiescent cells have been found to cause axonal injury and arrest neurite outgrowth in vitro. We have found that axonal injury causes c-Jun expression in a manner similar to that described for beta-actin. Coating the axons with C-Jun alone was constitutively expressed and increased during axon outgrowth. Using the 14-3-3 family of proteins, we measured the effects of hypoxia on c-Jun expression for the first time. The 14-3-3 protein was found to bind to c-Jun and to enhance c-Jun expression following hypoxic conditioning. In conclusion, hypoxia induces c-Jun expression and increases c-Jun expression following axon outgrowth.

617.20 Trk B IN RAT SPINAL MOTONEURONS AFTER SClATIC NERVE LESION.
L.A. Campbell* & I.-G. McQuarrie* VA Medical Center, and Case Western Reserve University, Cleveland, OH 44106 Neurotrophins act as trophic factors for specific cell groups in the nervous system during development and regeneration. The proto-oncogene receptors mediate these effects. Studies that localize trk B mRNAs in specific groups of neurons have been performed using in situ hybridization. We have used a trk B polyclonal antibody for immunohistochemistry to localize trk B receptor protein in the rat spinal cord. To study effects of axotomy, we transected the L4/5 spinal nerves (with contra-lateral sham lesions), and retrogradely labelled motoneurons with Fluoro-Ruby. Immunohistochemistry was performed with a biotinylated secondary antibody conjugated with horseradish peroxidase, using a methyl green counterstain. Preliminary data at 4 days post-axotomy showed an increase in the number of trkB immunoreactive motor neurons on the axotomised side, consistent with the localisation of fluorescence. This increase in trk B receptor protein above basal levels corresponds to known increases of its ligand, BDNF, in target tissues. (Supported by grants from the DVA.)
618.5

NOVEL INHIBITORS OF ASTROCYTE GLUTAMINE SYNTHETASE. K.I. Farrel,* D.K. Willis, and P.A. Sayegh, Dept. of Neurology, Univ. of California and VAMC, San Francisco, CA 94124, and USARADC, Dept. of Pharmacology, Univ. of Wisconsin-Madison. Glutamine synthetase is found in glia but not neurons. L-methionine-7-d-13C-glutamine (MSO) has proven useful in the study of glial functions as an inhibitor of glutamine synthetase. However, MSO has many other effects that may be unrelated to the inhibition of glutamine synthetase. This study identified alternative compounds that can inhibit glutamine synthetase. Inhibition of free enzyme prepared from sheep brain (Sigma) was determined by the method of Meister. Inhibition in primary rat cortical astrocyte cultures was also assessed using a 5 hour incubation of intact cultures with these compounds followed by washing and cell lysis.


drug

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (mM)</th>
<th>IC50 (mM)</th>
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<tr>
<td>methionine sulfoxide</td>
<td>91.6 ± 1.2</td>
<td>15.1 ± 0.1</td>
</tr>
<tr>
<td>glutamine</td>
<td>10.5 ± 2.2</td>
<td>0.005 ± 0.002</td>
</tr>
<tr>
<td>lactate</td>
<td>&gt;2000</td>
<td>7.0 ± 0.8</td>
</tr>
<tr>
<td>4-aminoacetone acid</td>
<td>400 ± 65</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>oxetn</td>
<td>212 ± 2</td>
<td>&gt;2000</td>
</tr>
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Inhibitor concentrations of up to 2mM did not cause astrocyte death, as assessed by lactate dehydrogenase activity of the cultures 24 hours post-incubation. Previous work with MSO has suggested a link between astrocyte glutamine and glycolysis metabolism. The effect of the novel inhibitors on astrocyte glutamine accumulation was assessed in astrocyte cultures derived from Sprague-Dawley. Each of the agents that inhibit astrocyte glutamine synthetase were also found to increase astrocyte glutamine, suggesting a causal relationship. These agents may be useful alternatives to MSO for the study of glial functions.

618.6

INHIBITION OF PROTEIN SYNTHESIS PREVENTS INTERLEUKIN 1a (IL-1a)-INDUCED INCREASES IN PROSTAGLANDIN (PG) PRODUCTION IN OVINE ASTROGLIA. C. Olson, B. Wall, and J. Bush, Dept. of Physiological & Pharmacological, Bowman Gray Sch. of Med., Winston-Salem, NC 27157-1083.

Little is known concerning actions and mechanisms of cytokines on prostaglandin production in astroglia. The purpose of this study was to examine short term (1-4 hour) effects of IL-1 on PG production in astroglial cultures from fetal sheep. We tested the hypothesis that increased PG production is dependent upon continued or enhanced protein synthesis. Immunofluorescence astrogliosis from second passage fetal ovine cortex were grown to confluence in 12-well (22mm) plates. PG~F~ production in medium determined using enzyme immunosay. Cells were exposed to 10 ng/ml IL-1 in medium or to 10 ng/ml IL-1 without the production of PG~F~ by 1586 ± 303 to 2688 ± 393 ng/ml at 2 hours (n=29; p<0.05) and from 1356 ± 369 to 5426 ± 1571 ng/ml at 4 hours (n=20; p<0.05). Coaplication of an inhibitor of prostaglandin H synthase (indomethacin, 10 µM, n=8) or of phospholipase A2 (arachidonic acid, 103M; n=8) prevented increases in PG~F~ production, as did H7 (10µM, n=5), an inhibitor of protein kinase C. Further, coinulation of IL-1 with actinomycin D (1µg/ml, n=9), a RNA synthesis inhibitor and cycloheximide (10 µg/ml, n=9), an inhibitor of protein synthesis, completely blocked the increase in PG~F~. We conclude that IL-1 increases PG production in ovine astroglia via a mechanism involving several steps, including activation of protein kinase C and phospholipase A2, and continued or enhanced protein synthesis. Supported by HL 30260 and HL 45558.

618.7

IDENTIFICATION OF VOLUME-SENSITIVE ORGANIC OSMOTIC CHANNELS IN HUMAN GLIAL CELLS. P.S. Jackson, K. Koga, and J.E. Meldrum, Div. Neuurosurgery and Medicine, Children's Hospital, Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

Regulation of cellular volume is a fundamental process critical to the survival of all cells, particularly those in the central nervous system where even minor volume changes can cause severe neurological damage. Over the past several years much has been learned about the basic biology of cellular volume control. It has now become clear that while osmotically sensitive pathways such as the mammalian p50 oligopeptide (mammalian lymo-protin) may play a central role in brain volume homeostasis. During periods of cellular shrinkage these oligopeptides are released from cells through a shared transport pathway which is now known to be a relatively non-selective Volume Sensitive Organic Osmolyte / Arson Channel (VSOC) 

618.8

CHARACTERIZATION OF VIP-INDUCED PROTEINS AND mRNA IN MOUSE ASTROCYTES BY 2-D GEL ELECTROPHORESIS AND BY mRNA DIFFERENTIAL DISPLAY (DDH). P.M. Magistretti, G. Pellegrini, C.L. Bolis and I. Cardinaux, 1Institut de Physiologie, Universite de Lausanne, Lausanne, Switzerland. 2Department of Biology, University of Milan, Italy.

In primary cultures of mouse cerebral cortical astrocytes, a rapid glycolysis inhibition followed by a massive glycerol synthesis (bic- to ten-fold) over basal levels after 1 hr treatment by VIP or norepinephrine (NA). Both actions of the neurotransmitters are mediated by VIP. Since the induction of glycerol synthesis triggered by VIP or NA is abolished by inhibition of protein synthesis and translated into the 2 gel electrophoresis and the mRNA DD techniques to search for newly synthesized astrocyte gene products induced by VIP or NA. The comparison of VIP-induced proteins from primary astrocyte cultures treated with VIP or NA revealed that only VIP is able to induce the expression of a protein (kDa M) in VIP 100 mRNA that was identified as an astroglial, a protein inhibitor, which is partially inhibited in astrocytes, is partially inhibited in astrocytes at the 35 kDa mRNA level. Using this technique we have observed several differences between the patterns of mRNAs from untreated cultures and from cultures exposed to VIP 1 µM or NA 100 µM. This technique may be used to identify differentially expressed mRNAs from astrocytes treated with VIP or NA expressing the VIP mRNA identified by DD.

618.9


Burnstock, Barnard and colleagues (Cell Biol. Lett. 32A, 219-225, 1993, 3IPS, 199-70, 1994) have reported cloning a G-protein coupled receptor from central nervous system that is activated by isoprenoids, a neurotransmitter (with 3-methyl-ATP-3ATP-ADP-UTP, but not ATP). As a corollary to the study of neuronal novoceptors, we have investigated ATP receptors on astrocytoma cells. Astrocytes were harvested from rat frontal cortex tissue and grown in culture on dishes for 21-28 days. Total RNA was extracted from primary cultures using the guanidinium method and poly(A)* enriched RNA purified with oligonucleotide (OLigD) and streptavidin-

618.10


Levels of ammonia and endogenous benzodiazepines (BDZs, eg. H) are increased in hepatic encephalopathy (HE). Since astrocytes are involved in HE, and since Kc uptake in astrocytes plays a major role in HE, it was examined the effects of the BDZs on various astrocyte neurotransmitters (products of peripheral-type BDZ receptor activation) in Kc uptake. Rat astrocytes in primary culture were incubated with 3-pi/ml of BDZ (100 µg/ml) for 1 hr in the presence of BDZs and neurotransmitters with or without 0.5 mM MgCl2. Ammonia alone inhibited Kc uptake by 26%. Agents that interact with the peripheral-type BDZ receptor (eg., R5-4844 and K11195 at 30 µm inhibited Kc uptake by 25%, whereas interferes with central-type BDZ receptor (clonazepam and flumazenil at 30 µm did not affect Kc uptake. The effect of K5-4844 on Kc uptake was significantly enhanced by 30 µm of BDZs, whereas the presence of 5 mM MgCl2 (35% inhibition). Fragnenoluc sulfate (50 mM) and 5a-pregnan-3a,21-diol-20-one (5a-THDOC; 30 µm) inhibited Kc uptake by 30 and 50%, respectively. The effects of neurosteroids were enhanced in the presence of ammonia (41-50% inhibition). Thus, astrocytes in HE are able to effect astrocyte Kc homeostasis which may result in altered cell excitability. (Supported by the Veterans Administration and USPHS grants AM-38153 and NS-30291, and GREEC)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
618.11 EFFECTS OF AMINOSTILZIDEPHENETHIOLS IN ADENOSINE UPTAKE IN ASTROCYTES. B.S. Dobrovy, A.B. Bander, and M.D. Rosenthal. Dept. of Pharmacol. and Toxicol. Univ. of Miami, Miami, FL 33101.

The mechanism of neuroinhibition in hepatic encephalopathy is unknown. Benzodiazepines (BDZs) are elevated in HE and are known to reduce the uptake of adenosine, an inhibitory neuromodulator. This study was undertaken to establish whether BDZ receptors on peripheral and central BDZ products, receptors of peripheral BDZ receptor activation (neurosteroids), as well as adenosine (elevated in HE), affect adenosine uptake. Rat astrocytes in primary culture were incubated with 1 μM [3H]-adenosine (0.7 μCi/ml) for 30 min in the presence or absence of the adenosine analogs. Adenosine uptake was inhibited by 9% in the presence of 5 nM [3H]-adenosine; however, 2-3 days of prior treatment with adenosine resulted in a 20-25% decrease in adenosine uptake. Agents that interact with the peripheral (Ro-44-6066 and PA 1179) and central (clonazepam and flumazenil) BDZ receptors at 10 μM, reduced adenosine uptake by 30%. Endogenous BDZs, DBI (40 μM) and D89 (10 μM), inhibited adenosine uptake by 15%. Flumazenil (10 μM) and 3a,4-mepenzolate 21-diol 20-one (3a,4-MEBC; 30 μM) also decreased adenosine uptake by 20% and 64%, respectively. Suppression of adenosine uptake by amanin, BDZs and neurosteroids may contribute to the neuroinhibition in HE. (Supported by the Veterans Administration and USPHS grants MA-38153 and NS-30291, and GREC).

In culture, microglia and astrocytes can express the inducible Type II form of nitric oxide synthase (NOS). It has been suggested that glia may contribute to neuronal cell death associated with cerebral ischemia and/or other neurodegenerative diseases by generating nitric oxide (NO) (Proc. Natl. Acad. Sci. U.S.A. 98:10945-10949, 1992) via this enzyme. Since microglia and astrocytes represent two distinct populations in brain, we investigated nitrite levels and the production of L-[14C]arginine from L-[14C]arginine. Increases in Type II NOS mRNA and nitrite levels were detected in astrocytes. Microglia and astrocyte cultures were exposed to a variety of stimuli, including bacterial lipopolysaccharide (LPS), gamma interferon, tumor necrosis factor alpha (TNF-α) or IL-1β. A combination of these factors. The culture was assessed for NOS activity by measuring nitrite levels and the production of L-[14C]arginine. Quantitative differences in NOS activity were noted when both cell types responded to identical stimuli. However, microglial cultures did not respond to concentrations of LPS alone that were stimulatory for the astrocytes. These results suggest that in vivo microglia and astrocyte NOS activation may be regulated differentially under different physiological or pathologic conditions.


We are studying factors regulating the expression of major histocompatibility complex (MHC) II antigens on rat astrocytes and microglia to better understand the role of these immune-related molecules in various neurological functions. We find that a significant proportion of the class II expression in the brain is poorly understood; the induction of these molecules during a number of neurodegenerative diseases indicates a possible role in the progression of the disease. Astrocytes and microglia were isolated from neonatal rat pups and stained for surface expression of H-2d or transacted with a plasmid containing the human MHC class II promoter (H-2dADA) driving a chloramphenicol acetyltransferase (CAT) gene (astrocytes only) to examine promoter function. Cells were treated with interferon-γ (IFN-γ) for 48-72 h. Northern analysis of RNA expression, in combination with a number of antibodies of intracellular signaling pathways. Preliminary data indicate induction of surface expression on astrocytes by IFN-γ can be inhibited by blocking protein kinase C activity, PKC inhibition also resulted in a reduction in microglial class II surface signal although expression was not retained above background. Our findings also show that selective inhibition of protein tyrosine kinase fails to inhibit IFN-γ-driven class II expression in either cell phenotype. Parallel experiments with transfected cells produced similar patterns of expression from the human promoter. This work suggests MHC class II expression on astrocytes and microglia may be under similar regulatory control with PMTs and some tyrosine kinases but may differ in their sensitivity to external stimuli.


When adult mammalian brain is injured, there is a complex response of non-neuronal cells which includes cell proliferation generating astrocytes in the affected region. The extent of the astrocytes mediated gene transfer to identify the lineage and characteristics of cells proliferating after injury. Knockin mice were generated to the rat cadherin protein to kill neurons, and four hours later L21 BAV virus was injected at the same site, to infect proliferating cells and transfer the reporter gene β-galactosidase. These were used immunocytochemically for the β-galactosidase immunoreactivity to detect proliferating cells in the tissue at various intervals after the injection of virus. This approach circumvents the technical problem that cytoplasmic localization of β-galactosidase activity may obscure immunoreactivity detected with immunofluorescence methods.

Forty eight hours after viral infection, most of the β-galactosidase-immunoreactive cells lacked β-gal immunoreactivity. In many instances, the shape of cells with β-galactosidase immunoreactivity was similar to that of cells expressing β-galactosidase, a marker of microglia. We are now examining the β-galactosidase-immunoreactive cells for the presence of microglial and macrophage cell markers such as ED-1 and ED-2 at several survival intervals after injection of the retrovirus.

618.20 ION CHANNELS AND OXYGEN RADICAL PRODUCTION IN ACTIVATED MICROGLIA. B.A. Ballyk*, D.J. Phipps, P.S. Pennathur and J.C. Schlichter. Playfair Neurosciences Unit, Toronto Western Hospital, Toronto, Canada, M5T 2S8.

The objective of the present study was to characterize ion channel expression in activated microglia and their ability to release oxygen radical.

Primary mixed cultures from the brains of newborn Wistar rats were starved to eliminate neurons and then shaken to isolate microglia, which were plated either in 96-well plates for functional assays or on glass coverslips for patch-clamp recording. Cultures which received conditioned media containing colony stimulating factor 1 (CSF-1) showed marked production of the kDa protein, which was measured by immunofluorescence. Immunocytochemical staining with 100 μM TMRM. Whole cell recordings showed that activated microglia expressed at least three ion currents: an inward rectifier K+ current blocked by external Ba2+, a delayed-rectifier K+ current with several features of the cloned K(Ca) channel, blocking by charybdotoxin and margatoxin, and a Cl- current blocked by furosemic acid.

In functional assays, microglia activated with opsonized zymosan produced superoxide and nitric oxide, as measured by reduction of cytochrome c and Griess reagent, respectively.

We are now using ion channel blockers in functional assays to determine what roles these channels play in the physiology of microglia.

Supported by the Savoy Foundation, the NHRDF, and the MRC (Canada).

GNE STRUCTURE AND FUNCTION V


Inherited variations of human catechol-O-methyltransferase (COMT) activity levels are controlled at a two allele, autosomal locus. The "low" activity enzyme is also relatively thermolabile, compared to the "high" activity form. A single amino acid change at position 47 of human C0M, in an adenine of H/L individuals and a guanino in most H/H individuals has been identified. PCR-amplified coding regions from L/L and H/H specimens were subcloned into the pGEM-3Z vector and protein was expressed using the Tnt 1 lysate coupled transcription/translation system (Promega). Mg2+, methionine-labeled proteins were separated by SDS-PAGE followed by autoradiography. Two major protein bands of 25.5 and 29 kD, observed in lane corresponded to soluble and membrane-bound forms of the COMT enzyme. SDS-PAGE analysis of these bands, following immunoprecipitation with antiserum to soluble COMT enzyme, revealed that the relative intensities of radioactive bands increased in the order of H/H specimens. Scanning densitometry indicated that the relative amount of radioautographic immobilizable protein in the 25.5 kD band from the H/H specimen was 3.9 times greater than those from the L/L specimen. This is in excellent agreement with measured liver COMT activity levels in these two specimens. These findings are supported by results on Western blots of human liver homogenates from individuals with inherited L/L and H/H genotypes. It can be concluded that the single base change in the COMT coding region influences regulation of expression of the human COMT gene either at the transcription, translation or degradation of protein.


We have characterized a mutation in Belgian sheepdog that disrupts the formation of the optic chiasm (Williams et al., 1994). The pattern of inheritance over 6 generations indicates that the mutant allele is autosomal recessive (symbol NOX, no optic chiasm). To identify NOX we have taken complementary approaches. Candidate genes encoding mutations at the tyrosinase gene locus (TYR) are known to perturb ganglion cell projections at the chiasm. We have used primers derived from conserved sequences of human and mouse TYR to clone exon 1 of dog homolog homology analysis of the polymerase chain reaction. The relationship between TYR and NOX was determined by sequence comparison and linkage analysis. Chromosome mapping: We have begun to map canine genes that are linked on mouse chromosome 7 over a 250 cM interval. This interval, located between the p locus and the calcium gene, contains several loci that play key roles in brain development and function. The TK6 assay is based on the TK6 assay, which is used to identify linkage disequilibrium in the human population. The F1 progeny were backcrossed to mutant Belgians. DNAs from the backcross offspring are being typed using several forms of markers, including restriction fragment length polymorphism, polymorphic microsatellites, and RAPD. This will allow us to begin constructing a molecular framework for the NOX locus, and for screening a dog genomic library to obtain probes that will be used to assign chromosomal position amongst linked loci via fluorescence in situ hybridization.

Support: NEI ROI-9586

Somatic gene transfer has been extensively investigated using genetically modified primary fibroblasts as grafts for intracranial transplantation in our laboratory: We use defined retroviral vectors to investigate gene expression and regulation in the adult central nervous system (CNS) on a molecular and cellular basis. Our data suggest that expression from retroviral promoters/enhancers decreases with the onset of quiescence in vivo and in grafted fibroblasts in vivo. When genetically modified fibroblasts are implanted into the host CNS, the lack of tumor formation indicates that the cells reside in a quiescent status within the grafting area. As a consequence, it is necessary to enhance the capacity of engineered fibroblasts to express transgene products in a quiescent state, and the objective of this project is to examine strategies of achieving long-term stable and/or regulatable transgene expression in primary fibroblasts in vitro and following implantation into the adult rat CNS. To achieve this goal we use several strategies: 1) The effect of different viral, tissue-specific and housekeeping promoter/enhancer configurations in vivo is assayed in growing and confluent cells in vivo. Fibroblast cultures in different serum levels have been established that may mimic conditions in the in vivo grafts. The effect of the size and composition of different transgenes on their expression is analyzed under the same conditions. 2) We also analyze whether potentially negative regulatory elements can be deleted from viral promoters/enhancers to direct transgene expression constitutively. 3) The influence of exogenous factors on transgene expression is analyzed for full-length as well as truncated promotor/enhancers. In addition to in vitro experiments on long-term stable and/or regulatable expression from various promoters/enhancer combinations is also monitored in vivo after grafting. Our results suggest that retroviral gene expression can be regulated by deoxynucleosae and thymine acid in vivo and in vivo.


We have adopted a technique of differential display of PCR-amplified mRNA to examine the nature and extent of changes in gene expression in mammalian brain. This technique utilizes a set of oligonucleotide primers, one anchored to the poly A' tail of the message and the other located upstream of the poly A' tail. While this technique has previously been used to study changes in cultured cells, little information is available on the application of this technique to heterogeneous tissues such as the brain. Currently, we are using this technique to examine changes in gene expression associated with seizure activity and focal cerebral ischemia. In the first example, we have isolated mRNA from animals that have been kindled to stage 5 seizures and left seizure-free for at least 2 weeks or from animals acutely treated with kainic acid. Electrotiled implanted or vehicle injected animals served as a sources of control mRNA. In the second example, we are studying changes in gene expression associated with a model of focal cerebral ischemia based on the photoinduction of thrombin stroke using the dye Rose Bengal. We have identified a number of differentially displayed messages in the brains of animals that have been kindled or have experienced kainic acid-induced seizures. Differential displayed messages have been isolated, PCR amplified, and subcloned into plasmid vectors. We are attempting to use both PCR-amplified material directly and subcloned material in Northern blotting experiments to further characterize gene expression associated with these differentially displayed messages in control and experimental animals. It appears that this technique is useful for studying changes in gene expression in the brain.

Supported by the Medical Research Council of Canada in partnership with SmithKline Beecham Pharma Inc. (Canada).

619.7 FACTORS AFFECTING AVAILABILITY OF ANTISENSE OLIGONUCLEOTIDES IN BRAIN. Y. Yaida, W.A. Pulsinelli* and T.S. Nowak, Jr, Dept. of Neurology, Univ. Tennessee, Memphis, TN 38163.

The use of antisenese oligonucleotides (oligos) to interfere with the expression of specific proteins is of demonstrated utility in vitro, and several reports have indicated that this approach may also be applicable to the intact brain. Few investigations have explicitly evaluated the localization of administered oligos within the brain. In the present studies we have examined the distribution and stability of oligos after intraventricular (i.e.) and intrahippocampal (i.h.) injection in the rat. These studies used synthetic oligos (19–25mers) antisense to various regions of a rat hsp70 mRNA sequence. Oligos were 3'-end labeled with [32P]thio-dATP, and 50 nCi (1 pmol, 2-5 μl) injected to examine distribution 1-24h after administration. Frozen sections (15 μm) were cut and subjected to film autoradiography. Localization and stability were evaluated 6-48h after injection of unlabelled oligos (2μmol) by in situ hybridization with 35S-labeled sense sequences.

After i.c. injection labeled oligo was largely periventricular. A more propagated signal was seen at 6 h or later, especially in white matter tracts. This likely does not represent intact oligo, since in situ hybridization detected only periventricular accumulation. With direct i.h. administration labeled oligo accumulated along the hippocampal fissure, probably reflecting injecting bulk flow. There was occasional prominent labeling of dentate granule cells, and limited CA1 labeling was also seen along the tract of the injection. Hybridization at 6-48h detected neuronal uptake of oligo with a similar distribution, including mossy fibers remote from the injection site. Unmodified oligos can therefore accumulate in neurons after local administration.

619.8 DEVELOPMENT OF PCREB AND FOS-LI IN SUPRAOPTIC NUCLEUS AFTER SALT LOADING. P. Shromn*, M. Magner, S. Winston, M. E. Charness, Dept. Psychiatry, Neurology, Harvard Medical School, VA Medical Center, West Roxbury, MA 02132.

Phosphorylation of the cAMP response element binding protein (CREB) precedes the induction of immediate early gene expression. Using antibodies that detect CREB from phosphorylated (CREB-P) and non-phosphorylated (CREB) CREB, we studied the appearance of CREB-like immunoreactivity (CREB-LI) and Fos-LI in the hypothalamic supraoptic nucleus (SON) in response to hypertonic stress in rats. Increased numbers of PCREB-LI cells were present at 15, 45, and 90 min after injection with normal or hypertonic saline. The number of PCREB-LI cells did not differ significantly between the two groups and returned to normal after 6 hrs. The SON of hypertonic saline-treated rats showed higher levels of c-fos mRNA than that of normal saline-treated rats, and only minimal signal was detected in the SON of unrestricted rats. The number of Fos-LI cells increased dramatically at 45 and 90 min after injection of hypertonic saline and did not change at 15, 45, or 90 minutes after injection of normal saline. The discrepancy between levels of PCREB-LI and c-fos mRNA suggests that hypertonic stress may activate additional transcriptional factors besides CREB. The lack of Fos-LI in the presence of modest increases in c-fos mRNA in normal saline-treated rats implies that levels of c-fos mRNA must exceed a threshold before increases in Fos-LI cells are detectable in the SON. Such a threshold might permit neuronal cells to activate diverse cAMP or calcium-responsive genes, through phosphorylation of CREB, without inducing the constellation of Fos-responsive genes.

Supported by AA06666, NS20146, Department of Veterans Administration.
619.9  
**Somatostatin decreases somatostatin messenger ribonucleic acid levels in the rat periventricular nucleus.** M. Aguilar. Department of Physiology, UT Southwestern Med. Ctr., Dallas, TX 75235-8873.

Somatostatin (SRIF), the inhibitory hypothalamic peptide of pituitary growth hormone secretion was reported to inhibit its own release by a negative feedback mechanism. However, it is not known whether this negative regulation is exerted at the molecular level. Therefore, this study was conducted to examine the hypothesis that hypothalamic SRIF mRNA levels might be regulated by its own protein. Periventricular nucleus (PVN) mRNA and protein were measured in rat brain using in situ hybridization in Waymouth's medium with SRIF or the SRIF analog RC 160 (10^-7 to 10^-3 M) for 6 h. Levels of SRIF mRNA were determined by a [35S] nucleic acid protection assay using a [32P]-labeled rat SRIF riboprobe. SRIF (10^-7 M) significantly (p<0.01) decreased SRIF mRNA levels. Likewise, RC 160 (10^-7 to 10^-3 M) significantly (p<0.05, p<0.01 respectively) diminished SRIF mRNA levels. These results suggest that SRIF can regulate its own gene expression by a negative loop feedback. SRIF secreted from these neurons may be down-regulating a preceding stimulatory input. Supported by NIH grant NS26821.

619.11  

Factors that regulate transcription of RNA and gene expression of voltage-dependent ion channels were identified using cultured N1E-115 neuroblastoma cells as a model system. Na and K channel-specific mRNA was measured using a competitive-polymerase chain reaction technique following reverse transcriptase of isolated total RNA. Amplified cDNA was sequenced to confirm identity of the transcript, and the absence of genomic DNA was verified. Cell division can be arrested resulting in an enhanced differentiated phenotype during incubation of cells in medium having reduced serum and 1.5% dimethyl sulfoxide. A reduction in Na channel mRNA was observed from adult male rats incubated in Waymouth's medium with SRIF or the SRIF analog RC 160 for 48 h. Therefore, Na and K content of these neurons is differentially regulated. Mechanisms responsible for the alteration in Na channel mRNA by Ca loading were studied. Although growth of differentiated cells in high external K did not alter mRNA level by itself, it blocked the decrease in mRNA in the presence of A23187. Na channel mRNA was reduced when cells were incubated in 250 µM chlorophenylhydantoin-cAMP (CAcAMP). A reduction by CAcAMP was not observed in cells grown in A23187. These observations suggest that the effect of Ca on transcription is mediated by CAcAMP, and perhaps coupled to changes in membrane potential. Supported by the National Multiple Sclerosis Society.

619.13  

In response to stresses, such as elevated temperature, cells respond by increasing synthesis of a group of highly conserved proteins known as heat shock proteins (hsp). One approach to study the expression of constitutive (hsc) and heat-shock-inducible (hsp) members of the hsp70 multigene family in the New Zealand white rabbit. Using radioactive and non-radioactive (DIG) in situ hybridization protocols, both hsc70 mRNA and hsp70 mRNA were localized to hippocampal neurons in control animals. The role of the stress-inducible hsp70 mRNA species in neurons of the untrained animal is yet to be determined. Following 1 h of hyperthermia, glutal cistals (including astrocytes, oligodendrocytes, and microglia) within the hippocampal region, the overlying corpus callosum and the fimbria showed an induction of hsp70 mRNA. We performed lectin cytochemistry using QUA 1-8 from Griffonia simplicifolia to identify microglia and immunocytochemistry with anti-GAP-antibodies to identify astrocytes shown that hsp70 proteins are found constitutively within dendritic processes of Purkinje neurons of the cerebellum, we now analyze transport of hsc70 mRNA in neuronal processes. In addition, a time course analysis of hsp70 mRNA induction in glial cell types investigates transport of hsp70 mRNA in glial processes following hyperthermia.

619.14  

Calcium or calcium-like kinase II (CaMKII) regulates both pre- and postsynaptic functions. CaMKII is highly abundant in the postsynaptic densities of excitatory synaptic terminals. Transgenic mice homozygous for a null mutation of the c-A subunit of CaMKII have impaired formation of hippocampal long-term potentiation, long-term depression, and abnormal short-lived plasticity. Behavioral studies reveal profound deficits in both spatial and contextual learning (Silva et al., 1992; Silva et al., unpublished results). The present study used stereological techniques to examine the ultrastructural characteristics of the CA1 region of the hippocampus in the homozygous mutant mice. Pyramidal cell density, CA1 stratum pyramidale, was obtained using the physical dissector on serial 1.5 µm sections. Synaptic density was obtained from serial electron microscopic tracings of stratum radiatum in CA1 using the optical dissector. We demonstrate similar neuronal and synaptic densities in the hippocampi (N=2) and wild-type animals (N=3). No differences were observed in the number of synapses per neuron. Initial analysis of the size of the CA1 region reveals no differences between the two groups. However, one mutant mouse appeared to display spontaneous seizure activity and examination of the hippocampal anatomy revealed cell loss in all CA regions of the hippocampal. Supported by NIMH 33521, NSERC, Kwanian Nry. Spec. Res. Fnd., Whitehall Foundation and Klingenstein Foundation.

619.10  
**The transcription factor c-erbB is induced by VIP, PACAP and noradrenaline in mouse cortical astrocytes.** J.R. Cardinaux and P.J. Magistretti. Insitut de Physiologie, Université de Lausanne, Switzerland.

The CCAAT/enhancer binding protein (c-EBP) family of transcription factors belongs to a class of proteins whose DNA-binding domain involves a basic region and a leucine zipper that is described as C/EBP and then for the Fos/Jun and ATF/CREB families. High c-EBP concentrations were found in the nuclei of fully differentiated hepatocytes and adipocytes. Therefore, it was considered that regulation of energy balance may be channeled at least in part through C/EBP. Since we have previously described transcription dependent metabolic effects of VIP and noradrenaline (NA) in astrocyt.(Klingenstein 12:4923), we have examined the effects of these neurotransmitters on C/EBP protein levels. By Western blot analysis, we observed that C/EBP is rapidly induced by VIP 10 nM, by the VIP receptor antagonist PACAP 10 nM or by NA 1 µM in mouse astrocytes prepared from the cerebral cortex. The effect of NA can be mimicked by the β-agonist isoproterenol, whereas methoxamine as α-agonist is devoid of any effect. Moreover, the NA-induction can be antagonized by atenolol and not be prazosin, thus suggesting that the induction of C/EBP is mediated by β-subtype adrenergic receptors VIP, PACAP and NA therefore probably increase the C/EBP expression via the cAMP second-messenger pathways. To our knowledge this is the first demonstration of the induction by neurotransmitters of a member of the C/EBP family in a mammalian cell. It is therefore searching putative target genes whose expression is modified in response to the C/EBP induction.
619.15 EXPRESSION OF CALMODULIN mRNA IN THE DEVELOPING RAT BRAIN. E. Berry and J.R. Brown,* Department of Zoology, University of Toronto, Scarborough Campus, West Hill, Ontario, Canada, M1C 1A4.

In the rat, there are three genes which have been identified which encode calmodulin (CaM). Previously in our laboratory, two CNDRs have been cloned and characterized which correspond to mRNAs of 1.8 and 4.0 kb derived from the rat calmodulin gene. Both messages are present transiently in neurons in the rat brain. We have extended our analysis to examine expression of these CaM mRNAs during neural development in the rat. New antiserum reveals the presence of the 4.0 kb transcript increases in abundance from postnatal day 1 (P1) in the cerebral hemispheres (CH) and thalamus. In the superior colliculus (SC), the 4.0 kb mRNA decreases transiently from P1 to P10 and then declines in later ages (P15 and adults). Levels of the 1.8 kb message remain relatively constant over development, while the 4.0 kb message shows greater changes. Using in situ hybridization, we observe that CaM mRNA is localized to a discrete layer in the developing SC. By P5 and P10 expression is focused to neurons between the superficial gray and optic layers. In the developing CH, CaM I mRNA is localized in a punctate pattern in cortical layers II-IV. Closer examination of cells in these layers reveals that CaM mRNA is present in the cell body and dendritic processes. The timing of CaM I mRNA expression in the developing SC coincides with the period of removal of mistargeted axons from retinal ganglion cells, while the appearance pattern in the developing CH correlates to a period of synaptogenesis in the cortical layers. This work was supported by grants to Y.R. from NSERC, Canada.

619.16 Concerted regulation of GAD and GABA_A receptor family transcripts as a model system in the molecular physiology of CNS development. A. Somogyi, X. Wen, V. Dupuis and L.J. Rinzel.

Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892

GABA (γ-aminobutyric acid) acts as a neurotransmitter molecule in embryonic development of the CNS, distinct from the well-established function of GABA as a principal inhibitory transmitter in the CNS. Evidence for this role includes the early embryonic detection of GABA-evolved pharmacological responses involving GABAA and GABAB receptors, and promotion of neurite outgrowth. The molecular basis for the embryonic role of GABA lies in its expression from glutamic acid decarboxylase (GAD), and the presence of GABA receptors. We have implemented a quantitative RT-PCR approach to test hypothetical models for the transcript regulation during development of the rat spinal cord dorsal. Three GAD mRNAs, GAD65, GAD67 and EP10 were detected at E11 and steeply increased during an early embryonic period. GAD67 reached maximum expression at the end of embryogenesis, at levels 100x higher than initially detected. During the postnatal period, GAD67 increased to their adult level at one third of maximum. The alternatively spliced transcript of the GAD67 gene, EP10, reached a maximum before GAD65/67 and then steeply dropped in late embryonic development. EP10 expression correlated with the transition of spinal cord tissue from proliferative to differentiating. RT-PCR of 13 GABA receptor subunits revealed overall parallel expression to GAD. As a working model, we propose that production of GAD transcripts may influence a regenerative feedback loop, linking the product of GAD, i.e. GABA, to the induction of GABA and GABA receptor subunit mRNAs. We are currently testing this hypothesis using embryonic spinal cord cells grown in a serum free tissue culture on an fibrin substrate. These conditions enable the continuing differentiation of cells.

POSTSYNAPTIC MECHANISMS III


Stochastic simulations (Fieber et al. Science 288:494, 1992; De Koninck & Moynihan. J. Neurophysiol. 71:138, 1994) have demonstrated the existence of intrinsic quantaal variance (\(\sigma_q^2\)) at the peak of miniature postynaptic currents (mIPSCs) in spite of the postulated monovalent current injections at central synapses. \(\sigma_q^2\) depends on the relationship between activation kinetics and bursting properties of synaptic receptor channels. The occurrence of gaps during bursts or clusters of channel openings can significantly affect the channel current. We examined how \(\sigma_q^2\) and \(P_{pump}\), the probability that ligand-bound channels will be open at the peak of mIPSCs, are affected by modulators of channel kinetics such as intracellular calcium, phospholipases and kinases. We simulated mPCs based on cell-attached single channel data obtained in acutely isolated adult neurons. Cluster of channel openings were aligned by exponentially distributed random intervals based on the time to first opening of channels following brief agonist injections (Edmonds & Colquhoun, Proc. Roy. Soc. Lond. B 259:279, 1992).

For unitary currents with burst and onset kinetics such as those underlying GABA_A receptor meditated mIPSCs, the \(P_{pump}\) is high, and modulation of the intraburst kinetics has little effect on the peak mIPSC amplitude. In contrast, as previously reported, (Zabors, Science 255:470, 1992; Rosemund et al., Science 262:754, 1993), \(P_{pump}\) is much lower for NMDA receptor mediated mPCs. Thus, modulation of the intraburst kinetics will dramatically affect peak amplitude. The alteration in \(P_{pump}\) will lead to changes in \(\sigma_q^2\). As the coefficient of variation (CV) used for quantal analysis assumes a fixed \(\sigma_q^2\), variations in \(\sigma_q^2\) by modulators of channel kinetics may produce erroneous interpretations of changes in CV at certain synapses.

Supported by NS-17528, NS-10549, and by the St. W. Richardson Foundation.

Y. DeKre is a Fellow of the Canadian MRC. D.N.L. is a Hughes Predoctoral Fellow.

620.2 MONOSYNAPTIC GABA-ARECEPTOR INPUTS TO RAT RUBURAL NEURONS IN SLICES C. P.T. Tseng* and Y.-S. Fu. Department of Anatomy, College of Medicine, National Taiwan University, Taipei, Taiwan, R.O.C.

Mammalian red nucleus(RN) displayed a differentiation induced sprouting of excitatory and inhibitory terminals, but the locations of inhibitory neurons remain enigmatic. Here we used intracellular recording and anatomical tracing methods to study the inhibitory connections of normal RN neurons in slices. In some cases, bicucullin was used to reveal the morphology of recorded cells. They resembled Golgi-stained RN neurons. Recorded caudal cells were similar to identified rubrospinal neurons revealed by fixed tissue intracellular dye injection.

Stimulating electrode was placed in the mesencephalic reticular formation dorsolateral to RN(3mm), substantia nigra(SN), or ventral tegmental demarcation(VTD). Stimuli in VTD evoked no postsynaptic potentials indicating that the lack of a recurrent inhibition. Stimuli in dMRF(11/11 caudal and 8/10 rostral neurons) and SN (16/19 caudal and 19/20 rostral neurons) evoked a short latency hyperpolarizing PSP. This poststimulus temporal pattern evoked by depolarizing current injection and it was reversibly blocked by perfusion of 20 μM bicuculline. Blockage of this IPSP revealed no underlying depolarizing PSP indicating a direct activation of inhibitory neurons or their axons. Latencies of IPSPs evoked from both stimulus sites were around 1.1 ms consistent with the hypothesis of being monosynaptic PSPs.

Anatomically, anterograde tracer-Destain applied in the dMRF or SN of live slices labeled neurons with bouton-bearing axons extending into the RN. They were often found to oppose somata in the RN. Immunohistochemical staining shows that dMRF and SN neurons contain high level of the calcium-binding protein-parvalbumin, known to present in high concentration in GABAergic neurons. Thus anatomical and physiological data suggest that dMRF and SN neurons mediate a GABA_A/IPSP on RN neurons(supported by NSC of Taiwan, R.O.C.)

620.3 FUNCTIONAL CHARACTERIZATION OF THE CORTICO-NUCLEAR PROJECTION IN RAT CEREBELLUM IN VITRO D. Mouginot and B. H. Gathier*. Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland.

Neurons of the deep cerebellar nuclei (DCN) constitute the major output of the cerebellar. Intracellular recordings were obtained to characterize the inhibitory synapses formed by Purkinje cells (PCs) on neurons in the DCN using organotypic cerebellar cultures. These preparations exhibit the unique property that PCs grow axons de novo, forming an impressive in vitro synaptic interaction projection. Inhibitory amino acid receptor antagonists (CNQX, 20 μM; D-APV, 40 μM) and bicuculline (40 μM), DCN neurons spontaneously fired action potentials whose frequency was linearly correlated linearly on methacholine. Thus, DCN cells displayed a besting-type pacemaker activity under conditions of synaptic isolation. DCN neurons possess GABAA and GABAB receptors, as shown by bath application of GABA and bicuculline (100 μM). In the presence of CNQX and D-APV, field stimulation of PCs evoked graded IPSPs which were completely and reversibly abolished by bicuculline. IPSP amplitudes were not significantly reduced during fast repetitive stimulation, and the resulting hyperpolarization was not affected by the GABAB receptor antagonist CGP-35348. Trains of IPSPs (5 to 10 Hz) were able to significantly discount the pacemaker activity of DCN cells. In addition, experiments with bicuculline show that PCs exert a tonic hyperpolarizing tone on DCN neurons.


Burst firing of dopamine neurons in vitro is induced by the glutamate agonist N-methyl-D-aspartate (NMDA). The hyperpolarization bursts of cellular activity due to NMDA excitation by a cationic-sensitive pump (Johson et al., Science 258:665). We formulated and resolved a theoretical model of the burst mechanism of burst generation. We show that a number of bursted, inward NMDA-mediated current and the outward Na^+-current pump is sufficient to generate the oscillation (0.5 Hz) suppressing the burst. The region of negative slope in the I-V relation of the NMDA channel in the presence of Mg^2+ is indispensable for the occurrence of this burst. We suggested by Seunig et al. (Neurosci 58:201, 2001) we find that the Na^+-current and the Na^+-pump inhibitors are required: a soma where action potentials are produced and a dendrite where the slow rhythm is generated. The time scale of Na^+-P handling in the dendrite is typically tens of seconds. The Na^+-pump inhibitors transform bursting back into tonic firing. When the soma is voltage-clamped, slow oscillations in current, which are generated in the dendritic compartment, are still present. These results are in agreement with experimental observations. Insights obtained with our model may apply to other neurons where bursts which appears to involve NMDA channel activity.
620.5


The major excitatory transmitter glutamate activates several subtypes of glutamate receptors. In this study, we investigated the interaction of these subtypes in single neurons. We carried out voltage-clamp recordings using the conventional whole-cell patch-clamp technique from hippocampal neurons, prepared from adult rat hippocampi (7-15 days) and cultured for 13 days. Glutamate (100 μM), applied by rapid pressure ejection, induced a large inward current with prominent desensitization in Mg-free, glycine- and TTX-containing standard solution. The current induced by glutamate was paradoxically increased to 122 ± 2% (mean ± s.e., n=9) of control by an AMPA/kainate receptor (kA/kR) antagonist, CNQX (10 μM). The enhanced current was also increased in the presence of the NMDA receptor (NMDA/kR) antagonist, D-APV (200 μM). In addition, in mutant mice lacking functional NMDA-Rs, CNQX reduced the glutamate-induced current to 33 ± 3% (n=5) of control, indicating that the NMDA current was increased by about 80% in the wild-type animals by blocking kA/kR. The ratio of glutamate current at 3 sec to its peak amplitude was increased by CNQX, indicating that the NMDA current had been desensitized by kA/kR activation. In Ca-free solution, CNQX reduced the glutamate-induced current to 76 ± 2% (n=9) of control. In Mg-free solution, with Li or Cs substitution, CNQX reduced the glutamate current to 74 ± 4% (n=3). Thus AMPA/kainate current may affect NMDA current via Na influx, which eventually increases Ca levels by the NlCa exchanger. Similarly, the NMDA-component of glutamate current was also increased by 120 ± 5% (n=6) by a metabotropic glutamate receptor (mGlUR) antagonist, MCPG (200 μM). These results suggest that the NMDA component of glutamate current is inhibited by kA/kR and mGlUR activities.

620.7

NOVEL TIME COURSE OF TRANSMISSION AT A GIANT GLUTAMATE SYNAPSE IN RAT VESTIBULAR CEREBELLUM. D.J. Rososky, D. Magnasco, and N.T. SLCR. Department of Physiology, Northwestern University Medical School, Chicago, IL 60611.

Unipolar brush cells (UBCs) of mammalian vestibulo-cerebellum receive inhibition from a single mossy fibre in the form of an extremely fast excitatory synaptic current (12-40 μs). UBCs are immunogenic for GABA and glycine (Magnasco et al., Synapse 16:284, 1994), but immunosuppressive for glutamate. In the present study we have examined the properties of transmission at this synapse using whole-cell recording methods in this cerebellar slice maintained in vitro. UBCs in the OCL of rat cerebellum noduli and ovals were patch-clamped with Lucifer Yellow (LY)-filled pipettes, and stimulating electrodes were placed in the white matter to activate mossy fiber afferents. Confocal fluorescence imaging of their characteristic morphology was used to verify cell identity. LY filled the soma, dendritic brush, and revealed a branching axon that gave rise to 2-4 mossy-like rosettes in the GCL. In whole-cell recordings a biphasic excitatory postsynaptic current (EPSC) was observed in UBCs voltage-clamped at -40 mV in Mg-free saline. The fast component (t<sub>0.7</sub>=5 ms, t<sub>0.3</sub>=36 ms) was followed by an unusually slow inward current (t<sub>0.7</sub>=220 ms, t<sub>0.3</sub>=84 ms), both of which were evoked in an all-or-none fashion at identical stimulus thresholds. At 40 mM it was blocked by the AMPA/kainate receptor antagonist CNQX (10 μM). In Mg<sup>2+</sup>-free saline both fast and slow EPSCs reversed near 0mV with linear I-V relations between -80 and +50 mV. The pharmacology of the slow EPSC changed with development. In young animals (6-20 days) it was preferentially blocked by the NMDA receptor antagonist D-AP5 (30 μM) and t-thiourea-kynurenic (100 μM) and displayed non-linear I-V relations in the presence of external Mg2+. In contrast, it was blocked by CNQX (10 μM) and displayed no Mg2+-channal block in older animals (>30 days). It is proposed that the ultrastructural design of this synapse represents a specialization to prolong the lifetime of glutamate in the clef following release, thus resulting in rebinding of glutamate to produce a long-lasting EPSC whose time course is independent of receptor type.

620.8


While synaptic activation of NMDA receptors within the intrinsic circuitry of the vestibular nuclei (VN) has been suggested to contribute to plasticity of vestibular function, to date there has been no demonstration using intracellular recording methods of an NMDA receptor-mediated component of synaptic potential in vestibular nuclear neurons. In the present study we have re-examined this issue using whole-cell recording methods in 300-450-um thick slices of the medial vestibular nucleus (MVN) maintained in vitro. Slices were cut at the level of the vestibular nuclei (vVN), and stimulating electrodes were placed in the vVN to evoked EPSPs. In normal saline, the vVN-evoked EPSP was blocked by the NMDA antagonist D-AP5 (10 μM). However, in the presence of the NABAA receptor antagonist bicuculline (10 μM) a late, slow component of the EPSP was observed which was blocked by the NMDA receptor antagonist D-AP5 (50 μM). This monosynaptic NMDA-mediated component could be isolated in the presence of D-AP5, and displayed the non-linear voltage-gated characteristics of monosynaptic-mediated responses in the presence of external Mg. In some cells, D-AP5 also blocked a large (>50%) proportion of the δ-AP5-sensitive component. Polysynaptic AMPA/A-NMDA-activated EPSPs were observed in the presence of D-AP5. Prolonged bath application (10-20 min) of the metabotropic glutamate receptor (mGlur) agonist 15,3-ACPD (10-100 μM) produced a moderate, slowly desensitizing depolarization (2-3 mV) and an acute depression of the EPSP in all cells. A long-term potentiation of the NMDA-mediated EPSP was observed in 9/13 cells following the washout of 15,3-ACPD. These results demonstrate that NMDA receptors contribute both to the monosynaptic vVN-evoked EPSP and to transmission within the intrinsic circuitry of the rat MVN, and are modulated by activation of mGlurA.

620.9

SATURATION OF AMPA RECEPTORS FOLLOWING QUANTAL TRANSMITTER RELEASE. C.-M. Tang*. M. Margulis, Dept. of Neurology, Univ. of Maryland Sch. of Med.

While it is generally believed that postsynaptic NMDA receptors reach saturation following the release of glutamate from a single transmitter vesicle, it remains unclear whether AMPA receptors reaches saturation. Using biochemical and functional approaches in this study, we investigated the possibility that AMPA receptors do not follow Poisson behavior nor did they summate linearly. A minority but statistically significant number of closely timed msEPSCs were elicited from small numbers of release sites on proximal dendrites of cultured hippocampal neurons. In contrast, msEPSCs did not follow Poisson behavior nor did they summate linearly. A minority but statistically significant number of closely timed msEPSCs were also closely matched in terms of amplitude. Addition of cyclothiazide (100 μM) increased the non-linearity of summation. By statistical significance we refer to the "amplitude pairing". Receptor saturation best explains these observations. AMPA receptor saturation following quantal release has implications for synaptic transmission in the CNS.

620.10

DOES GLUTAMATE RELEASE SATURATE AMPA RECEPTORS AT A SYNAPSE? W.R. Holmes*, Neurobiology Program, Dept. of Biological Sciences, Ohio University COM, Athens, OH 45701.

The fact that few AMPA receptor channels are ever open at a synapse and that NMDA receptors have a high affinity for glutamate suggests that a single vesicle of glutamate might saturate all NMDA receptors at a synapse. In our previous model of LTP (Holmes and Levy 1990), the first few pulses of a 400 Hz input caused the peak number of NMDA receptors bound to glutamate to increase. However, if glutamate saturates NMDA receptors, this peak number should be the same for all pulses in the train. Whether or not the peak number of bound NMDA receptors increases during a tetanus affects model predictions of the amount of calcium entry through NMDA receptor channels. A diffusion model similar to that reported to simulate transmission synapses by Waluty et al. (1979) was constructed. A single vesicle of glutamate was assumed to be released either once or 8 times at 400 Hz. The numbers of bound and open receptors in AMPA and NMDA receptor channels are computed. Conditions when NMDA receptors would or would not be saturated were determined. For saturation of NMDA receptors to occur with the first pulse of a tetanus, a) the number of glutamate receptors per cell must be such that the peak number is much larger than the number of NMDA receptors and b) the first binding constant (k<sub>B</sub>) must be large. Under these conditions, a tetanus allows many more receptor channels to be occupied than through a single peak number of bound receptors never rises above that of a single input. The first binding constant has been estimated to be 5 ×10<sup>-1</sup> (Clements and Westbrook 1991). With this value, the number of NMDA receptors bound with glutamate increases with successive pulses of a tetanus, indicating a lack of saturation. This occurs even when the number of released glutamate molecules is 10 times the number of NMDA receptors.
SATURATING CONCENTRATIONS OF GLYCINE REVEAL UIBOJUTIOUS PRESENCE OF EXCITATORY SYNAPSES IN CULTURED HIPPOCAMPAL NEURONS R.S. Wilson and B. Mohk.

In the mammalian hippocampus, excitative postsynaptic currents (EPSCs) are mediated by activation of both non-NMDA and NMDA receptors colocalized at the synapse. Excitative postsynaptic responses in studies in which miniature EPSCs (mEPSCs) have been shown to consist of both non-NMDA and NMDA receptors. As an early report (Bekkers & Stevens, 1989) suggested that more than 70% of mEPSCs were in cultured hippocampal neurons, we examined the contribution of both components, whereas the remaining 30% could be evenly divided between synapses containing either non-NMDA or NMDA receptors exclusively. In the present study, we examined the contribution of each glutamate receptor subtype by making cell-attached recordings from the same terminals that were activated during evoked responses. We found that as the concentration of glycine was increased from 0 to 10 μM, the NMDA component of the evoked EPSC became significantly greater in both amplitude and duration. Furthermore, in 10 μM glycine, we saw no evidence of NMDA receptors comprising only of the non-NMDA component. Therefore, in our hippocampal culture preparation, there are no postsynaptic sites comprised exclusively of non-NMDA receptors. In addition, although there were mEPSCs comprised of only the NMDA component, it is not possible to conclude that non-NMDA receptors were not present, as desensitization of these receptors, as well as a weaker affinity than the NMDA receptor for the endogenous ligand, could mask their presence. NS24260 (MAD) & AG1200301 (RM).

DIFFERENCES IN INHIBITORY SYNAPTIC INPUT INFLUENCE EXCITABILITY OF CAT ORCHID GANGLION CELLS J. H. Gottlieb* and M. Chester

The occurrence of extracellular alkaline shifts during excitatory synaptic transmission (1) suggests that the NMDA receptor-H* modulatory site (2.5) may play a functional role. We amplified these pH shifts with benzoylacetone (BZ), a carbonic anhydrase inhibitor, and studied the EPSCs of whole-cell clamped CA1 neurons in rat hippocampal slices. In Mg2+-free, CO2/HCO3- media, 1 μM BZ caused an immediate increase in the EPSC amplitude at holding potentials of -80 to -40 mV (see Fig.) and a decrease in the EPSC amplitude at -140 mV. This was timed to avoid an amplification of the extracellular alkaline shift by +4.14 (CO2/HCO3- plus ATP (20-75 μM). The EPSC was unaltered by BZ, while the alkaline shifts were still increased (114 ± 23%, n=8). In HEPS, where buffering was independent of carbonic anhydrase, neither the EPSC nor the alkaline shift was affected by BZ (n=4). These data demonstrate that kinetics of the NMDA receptor-mediated EPSCs should be calculated using the buffering capacity of the extracellular fluid. The results indicate that endogenous pH shifts can modulate NMDA receptor function in a physiologically relevant time frame.

621.1

POTENT NAPHTHALENIC AGONISTS FOR MELATONIN RECEPTORS IN BRAIN. I.P. Niles*, I. Smith, J. Wang, J.J. Chen* and G. Fitzmaurice. Departments of Biomedical Sciences and ‘Nuclear Medicine, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

In studies aimed at the identification of selective ligands for melatonin receptors in the CNS, we have assessed the binding and functional activities of a series of naphtaleinic compounds bearing variable halogen substitutions on the N-acetylamo group. Binding experiments were carried out with the melatonin receptor radioiodide, 2-125Ijodozolomelatonin ([2-125]Izolomelatonin, [Izolomelatonin], in either whole-brain homogenates or synaptic fractions (Ps) fractions). Competition experiments performed on, in 30 mM Tris-HCl buffer (pH 7.4 at 4°C), revealed that these compounds compete with both (spectroscopically and low (nanomolar) affinity binding sites for melatonin. In assays conducted at room temperature in the presence of MgCl2 (4 mM), both binding components were also evident. More importantly, under these conditions, some naphtaleinic drugs exhibited a marked increase in their competitive potency at the high-affinity sites labelled by [125]Izolomelatonin, as indicated by affinities in the sub-picomolar range. Preliminary functional assays, in chick brain and rat hypothalamic membranes, show that these ligands inhibit forskolin-stimulated adenylyl cyclase activity. These findings indicate that compounds characterized by a naphtaleinic nucleus, a 7-methoxy group and halogen substituents on the N-acetyl side chain are high-affinity agonists at CNS receptors for melatonin. (Supported by a NSERC Strategic grant and SERVIER).

621.2

ENDODGIOUS GLUTAMATE INDUCES DEPHOSPHORYLATION OF THE NEURONAL CYTOSKELETAL PROTEIN MAP2. E.M. Quinlan and G. Herbison, Department of Neuroscience, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

Cytoskeletal proteins are likely targets of glutamate transmission in the nervous system. In collaboration with G.Herbison, the neuronal cytoskeleton, we have focused on the the microtubule-associated phosphoprotein MAP2 in rat hippocampal slices. Changes in MAP2 phosphorylation are known to regulate its interactions with microtubules and actin. Accordingly, multiple phosphorylation sites along the primary amino acid sequence represent potential targets for synaptic regulation of MAP2 function. Previous studies suggested that NMDA receptor activation is potently coupled to MAP2 dephosphorylation. Additionally, dephosphorylation of slices with 40 mM MgCl2 induced a rapid dephosphorylation of MAP2 by blocking the activity of endogenous glutamate. It is well established that glia possess sufficient glutamate uptake mechanisms, and that endogenous glutamate is released from slices by spontaneous neuronal activity. Application of 1 mM dilydrokainate, an inhibitor of glutamate uptake by glial cells, induced a rapid dephosphorylation of MAP2, suggesting that endogenous glutamate is a potent signal for MAP2 dephosphorylation. NMDA-induced dephosphorylation is dependent on extracellular calcium concentration. These data suggest modulatory dephosphorylation activation of the calcium-dependent protein phosphatase 2B (PP2B or calcineurin), which targets specific proteins, such as MAP2, for dephosphorylation.

621.3


Rules for MAP kinase in synaptic function have been suggested by (1) its occurrence in the brain, especially in the neuronal dendrites, and (2) its excitatory neurotransmitter, glutamic acid, and electric stimulation inputs activate MAP kinase. In this study, to clarify the function of the enzyme in the synapse, we surveyed MAP kinase substrates that occur in synaptic fractions. Supernatant and synaptic fractions (Ps) synaptosomes, synaptic plasma membrane (SPM) and postsynaptic densities (PSD) fractions were prepared from Wistar male rats. MAP kinases were purified from Xenopus oocyte or obtained from UBI (sea star p44/42 MAP). Protein kinase activities in each fraction were heat-inactivated before phosphorylation by the MAP kinases. Western blotting showed the occurrence of both MAP kinase and MAP kinase in all the fractions mentioned above. Fractions other than PSD contained bands immunoreactive to anti-MAP kinase antigen, these possibly corresponding to ERK1 and ERK2, respectively. ERK3 was greater than ERK1 in content in both synaptosomes and SPM fractions, and only ERK2 was detected in the PSD fraction. All the fractions contained substrates for both enzymes, but each showed a different substrate profile. Interestingly, sea star MAP kinase strongly phosphorylated the substrates in the supernatant and Ps fractions, while Xenopus MAP kinase (ERK2 type) activated more on the substrates in the synaptic fractions, especially those in the PSD fraction. Phosphorylation of the PSD proteins by Xenopus MAP kinases was increased after maturation of synapses. These results indicate that part of ERK2 occurs in the synapse, especially in the postsynaptic side, and that it may play some role(s) in the synaptic function via phosphorylation of synapse-specific substrates.

621.4

ACTIVATION OF NFkB UPON GLUTAMATE STIMULATION IN CULTURED MOUSE CEREBELLAR GRANULE CELLS. L. Guerrini, F. Blasi and S. Denti-Domin{it} Genetics and biology departments and Center of Cytopharmacology, Milan, Italy.

NFkB (the relA/p50 heterodimer) is a pleiotropic transcription factor. It occurs in an active form in the nucleus of a restricted number of cell types (lymphocytes); in most other cell types it is present in a covalently phosphorylated form and can be induced to move to the nucleus in response to a wide variety of extracellular stimuli. The localization of relA has been studied by immunocytochemistry in brain sections and in cultures of cerebellar granule cells. In brain sections, relA staining suggests a synaptic localization, as already described in the rat (Kalschmidt et al. Proc. Natl. Acad. Sci. 1993). In neuronal cells in vitro, relA is mostly concentrated at the tip of processes and at contact sites. This type of spatial distribution prompted us to investigate whether physiological stimuli, such as nanomolar concentrations of neurotransmitters, could activate NFkB in such cells. Glutamate pulses induce the nuclear translocation of NFkB, as observed by band shift experiments and this induction is specifically blocked by the NMDA receptor antagonist APV. With specific antibodies, we have assessed that the activated transcription factor is the heterodimer relA/p50. We are currently investigating the potential target genes of glutamate activated NFkB.

621.5


We have reported that sudden exposure of dissociated hippocampal neurons to strongly anisomotic or glucose deficient solutions suppressed voltage gated Na and Ca currents (Bainton et al., Brain Res., 632: 180-194, 1993). We now investigated whether GABA-induced current were similarly shut down by exposure to anisomotic solutions. Freshly dissociated rat CA1 pyramidal neurons were bath-clamped in whole-cell configuration. Voltage gated Na and K currents were pharmacologically blocked. The effect of hypo-osmotic, NaCl deficient (mannitol-substituted), hydro-osmotic test solutions delivered from a micropipette was tested on membrane currents evoked by brief pulses of muscimol and on voltage gated Ca2+ currents. Hyper-osmotic solution caused cells to shrink, but hypo-osmotic solution had no effect on cell size or shape. All current solutions depressed the muscimol-induced current and usually also the voltage-gated Ca2+ currents. V-gated Ca2+ currents were depressed by anisomotic, but not by NaCl deficient isosmotic solution. We conclude GABA receptor controlled ICl channels may be partially shut down in a strongly anisomotic or NaCl deficient environment.

621.6


In order to determine whether swelling of hippocampal tissue caused by lowering of osmotic pressure, hippocampal slices were exposed to bathing fluid from which varying amounts of NaCl was deleted. Interstitial volume ratio (ISV) change was determined from the volume of dialysis of the ions TMA+ or TEA+ administered by iontophoresis. Tissue electrical resistance was measured as the voltage drop generated by subliminal constant current pulses. The decrease of ISV determined from the dialysis of probe ions was dependent on the degree of tissue tonicity, and in the less severe condition ISV shrank to 3.3% of the total tissue volume. Prolonged hypertonic exposure revealed regulatory volume decrease (RVD). After restoring normal osmotic pressure, the tissue volume was increased in the volume of exchange of ions TMA+ or TEA+ administered by iontophoresis. All current solutions depressed the muscimol-induced current and usually also the voltage-gated Ca2+ currents. V-gated Ca2+ currents were depressed by anisomotic, but not by NaCl deficient isosmotic solution. We conclude that GABA receptor controlled ICl channels may be partially shut down in a strongly anisomotic or NaCl deficient environment. (Supported by CNPq, Brazil, the Hughes Med.Inst., & NIH grant NS17771)
262.1.7

The Serotonergic Inhibitory Postsynaptic Potential in Peripotential Hypoglossal Motor Neuron: Is It Reversibly Patched?

Synaptic inhibition mediated by the activation of potassium channels has been reported from several types of neurons. In each case, the K⁺ conductance underlying the synaptic potential is activated by a G protein and inwardly rectifies. We report here a second K⁺ current that contributes to synaptic inhibition. Intracellular recordings were made from guinea pig nucleus prepositus hypoglossi in vitro, where we have described a 5-hydroxytryptamine (5-HT)–mediated synaptic postsynaptic potential (IPSP). Voltage-clamp analysis of the current induced by applied 5-HT revealed two separate conductances: an inwardly rectifying, rapidly-activating K⁺ current (Ig) and an outwardly rectifying, slowly-activating K⁺ current (Icq). Ig was blocked by extracellular Ba²⁺ (200 μM) and TEA⁺ (126 mM). Icq was blocked by Cd²⁺ and intracellular BAPTA, indicating Ca-dependence.

Single focal electrical stimuli evoked a 5-HT-mediated IPSP, or under voltage-clamp, an inhibitory postsynaptic current (IPSC). Ba²⁺ blocked only a component of this IPSC, which corresponded to the current caused by Ig. When multiple stimuli were applied (to prolong the release of transmitter), the time-dependent current Icq was more fully activated, resulting in an augmentation of the IPSC. We conclude that the IPSC is caused by both currents and that its amplitude is a degree to which Ig is activated. This represents a mechanism by which synaptic responses can be potentiated.

262.9


The role of metabotropic glutamate receptor (mGlur) in modulation of phrenic motoneuron excitability was studied in the in vitro neonatal rat brainstem/spinal cord preparation. Spontaneous PMN activity was recorded from cervical ventral root (C4) or by whole-cell patch-clamp techniques. In order to activate mGlur, trans-15(1R,2R)-2-amino-4-cyclothiazepane-1,3 dicarboxylic acid agonist (ACPD) was applied locally via pressure ejection over the ventral spinal cord surface at the level of the phrenic nucleus. To block mGlur, (R)-2-methyl-4-carboxyphenylglycine (MCPG) was added to the bathing solution. Local application of ACPD (0.2 - 0.7 μM) induced a spike of the inspiratory activity, in a dose-dependent manner. At higher concentrations (0.5, 0.7 mM), tonic activity was superimposed on the inspiratory activity. MCPG (0.1-1 mM) effects of ACPD. Local application of ACPD depolarized PMN resting membrane potential (10-15 mV) and produced a concurrent 9% decrease in the inspiratory drive potential. Current pulse injection under control conditions induced several spikes after ACPD application. Under voltage-clamp conditions, ACPD caused a tonic inward current (-150 pA) and a 32% drop in amplitude of the inspiratory synaptic current. The I-V curve exhibited a 10% increase of input membrane resistance. The ACPD-induced inward current remained after TTX was added to the bathing solution and was partially blocked by MCPG (0.4 mM).

These results show a postsynaptic action of ACPD and suggest that mGlur can modulate the excitability of PMNs. Supported by NIH Grant NS24742 and the Conseil Régional PACA.

LONG-TERM POTENTIATION: PHARMACOLOGY I

262.2


Electrophysiological studies of drug actions typically assess direct membrane effects on monosynaptic responses in order to characterize cellular mechanisms of action. However, the ultimate effects of drugs on behavior may depend on the compound's actions across serial cortical circuits. Drugs that modulate the function of AMPA-type glutamate receptors are of interest since AMPA receptors mediate responses at most excitatory synapses in the brain. The present study was undertaken to compare the effects of a novel AMPA receptor modulator, CX-516, on mono- and polysynaptic responses in hippocampus.

Hippocampal slices were prepared from adult male rats using conventional methods. Stimulation electrodes were positioned to activate a pyramidal tract (PP) fibers in the molecular layer of the dentate gyrus and Schaffer-commissural (SC) fibers in CA1. A recording electrode was placed in the CA1 apical dendritic field. PP stimulation evoked small (0.1-0.3 mV) negative field potentials in CA1 with latency and waveform characteristics consistent with them being mediated by the triphasic hippocampal circuitry. SC stimulation was set to elicit polysynaptic responses of the same amplitude as the triphasic response. CX-516 (25 μM, n = 6) reversibly increased the amplitude of the monosynaptic SC-evoked field EPSP by 52% (±18, S.D.) and that of the triphasic response by 188% (±56).

These results suggest that CX-516 is a new tool for studying the role of AMPA receptors in facilitating long-term potentiation in vivo. (Supported by AFSOR, ONR, NIH, and the Academy of Finland).

Previous work has shown that an experimental drug (BPD-1L3, benzodiazepine-5-y-carboxyloxypropionate) increases the amplitude and duration of EPSPs recorded in slices of hippocampus, while having little effect on the slope of responses. Studies with outside-out patches indicate that BDP modulates AMPA receptor gated currents. After intraperitoneal (i.p) injections and for 2 hours, BDP influences monosynaptic responses in the dentate gyrus and area CA1 in vivo in a similar manner to that observed in slices. Here we tested the effects of BDP on LTP in the CA1 of freely moving rats. LTP was induced using a minimal stimulation paradigm involving pairs of short high-frequency bursts (paired theta bursts) repeated 5 times at 30 seconds; this pattern produces a small and transient (24h) increase in the drug effect. Each of nine animals was tested both in absence and presence of the drug in a counterbalanced fashion. Injection of the drug prior to theta burst stimulation greatly facilitated the subsequent induction of LTP, i.e., the increase in slope and amplitude was larger in magnitude and more persistent (several days) following drug than vehicle injections. Biodistribution and pharmacokinetics were examined with positive emission tomography (PET) using BDP radiolabeled with carbon-11; the results confirmed that the drug rapidly migrates from the injection site and crosses the blood-brain barrier; time curve analysis for brain, heart and liver showed that the drug reaches a maximum in the brain in less than 4 minutes. The facilitatory effects on LTP induction in freely moving animals were replicated with an analogue of BDP (BPD-5), which was also found to improve retention in a radial maze and in an odor matching problem. These results define the first tool for enhancing LTP in vivo and confirm an important prediction from the hypothesis that LTP is a substrate of memory.


Recent studies have identified a novel class of drugs (Ampakines) that facilitates excitatory synaptic currents mediated by AMPA receptors. Since aging is accompanied by decreases in excitatory communication, it has been suggested that such drugs might offset certain types of age-related deficits. This idea rests on two unexamined assumptions about AMPA receptors in the aged brain i) that the binding site(s) for the drugs is unchanged from that found in young brains and ii) that the channel kinetic properties affected by the drugs are also comparable in young vs. aged brains. These assumptions were tested by comparing drug effects on synaptic responses in young (3-4 months) and aged (24-28 months) rats. Hippocampal slices were prepared using conventional methods and field EPSPs recorded in slices (CX-516 and CX-517) found to potent effect potentials in pilot studies were used. Both drugs produced qualitatively similar, dose-dependent, reversible enhancements of field EPSP amplitude and prolonged decay times in slices from young and aged rats. CX-516 had more potent effects on response amplitude whereas CX-517 was more potent in modulating the decay time. Quantitative comparisons involving several response parameters and patterns of synaptic activation will be described and shown to reinforce the conclusion that the drugs have essentially the same effects on synaptic responses in the young and aged hippocampus. Based on these findings, it appears that Ampakines may be used to test the hypothesis that facilitation of fast, excitatory transmission will have positive effects with regard to age-related changes in brain function. (Supported by APOSF, ONR, NIA, and NIH).


Quantitative in situ hybridization and reverse-transcriptase PCR were used to determine the relative levels of AMPA receptor subunit (GluR1-3) mRNAs in rat forebrain. In all regions sampled, Glur2 cRNA hybridization was dense, being greater than or equal to the other two subtypes. Levels of Glur1 and Glur3 mRNA, however, varied markedly between hippocampus, neocortex and piriform cortex. In hippocampal neuronal layers, Glur1 cRNA hybridization density was equal to Glur2 but was 3-4 fold higher than Glur3. In contrast, parietal cortex (layers II/III) had relatively low levels of Glur1 (40% of Glur2), but high levels of Glur3 (60% of GlrU2). In piriform cortex (layer II), Glur1 and Glur3 mRNA levels were both low (33% of Glur2). If relative levels of mRNA are predictive of relative levels of subunit protein, then it is likely that the stoichiometry of the AMPA receptor varies substantially between synaptic systems in cortical zones of the telencephalon. Attempts to test this using immunoprecipitation with antibodies directed at the subunits of the receptor are in progress. It will also be of interest to determine if the regional variations described here are associated with differences in plasticity of the LTP type which is reported to be dependent upon the AMPA receptor subunit composition as well as with the effects of recently developed drugs ("Ampakines") that facilitate AMPA receptor mediated responses in the hippocampus of behaving animals. Supported by AG00538.

62.7 TEA PRODUCES A PERSISTENT ENHANCEMENT OF SYNAPTIC TRANSMISSION IN RAT NEOCORTEX. Marc R. Pelletier* and John J. Habibitz. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294.

We investigated the effects of teatetramyllanion (TEA) to induce long lasting changes in neocortical synaptic activity. Intracellular recordings were obtained from layer III/V pyramidial neurons in in vitro brain slices. Bath application of 25 mM TEA for 7 min produced transient effects on passive membrane properties and weak orthodromic input responses. However, input resistance was increased by 16 ± 4.2% of the duration action potentials, evoked by depolarizing current, was increased dramatically, an effect consistent with the blockade by TEA of K+ channels. Action potential duration was not different from control after 30-40 min of wash, TEA was then considered to have been washed out. TEA produced a persistent (45 min of wash) increase of 158% ± 7.6% in the amplitude of EPSPs evoked by weak orthodromic stimulation (layer IV/V, 0.05 Hz). The amplitude of EPSPs evoked by strong orthodromic stimulation was increased by 180 ± 10.0 %. These effects on synaptic transmission were APV (200M)-resistant (n=4). Orthodromic stimulation during TEA application was not required for enhancement (n=5). The enhancement of EPSPs, but not IPSPs, was blocked by recording with pipettes containing 200 mM BAPTA (n=4). TEA-induced enhancement of EPSPs in the neocortex is similar to that described in hippocampus. Additionally, we report an enhancement of IPSPs, which might be mediated presynaptically via an increase in GABA release.
622.9

In physiological conditions, the induction of LTP is mediated by NMDA receptors, whereas the expression is mediated primarily by AMPA receptors (AMPAR-LTP). To investigate the role of NMDA receptors in LTP, we have used the redox-sensitive probe 2,3-dinitrobenzenesulfenyl chloride (DTNB) to modulate the redox state of the NMDA receptor.

Our results show that the redox state of the NMDA receptor is crucial for the induction of LTP. In control slices, DTNB did not affect the expression of LTP. However, in slices treated with DTNB, the expression of LTP was completely abolished. This suggests that the redox state of the NMDA receptor is essential for the expression of LTP.

These findings have important implications for understanding the mechanisms underlying long-term potentiation (LTP) and may provide new targets for the development of anti-epileptic drugs.
622.15


PQQ is a putative essential nutrient shown in vitro to diminish NMDA ionic currents and neurotoxicity by direct oxidation of the NMDA receptor redox site (J. Neurosci. 1992;12). Administration of PQQ prior to hypoxia/ischememia induced rats. This effect was supported by a significant reduction in the slope of the EPSP (52.5 ± 8.5% (mean ± SE, n=12 slices, n=12 rats). DTT exposure (100µM for 30 min, followed by 30 min wash) did not affect the magnitude of LTP (88.9 ± 16.6% (n=8 slices, n=8 rats, p<0.04). In contrast, following exposure to DTT, PQQ (100µM) significantly reduced LTP (10.7 ± 4.7%, n=7 slices, p<0.005). These results are consistent with in vitro observations from cultured cortical neurons and demonstrate, in intact, that exposure to PQQ reverses the action of DTT on NMDA receptor mediated events (NS3718, EFA, AHA).

LONG-TERM POTENTIATION: PHARMACOLOGY I

623.1

THE INFLUENCE OF GABA, AND GABA-ANTAGONISTS ON THE MAINTENANCE OF HIPPOCAMPAL LONG-TERM POTENTIATION IN RATS. Klaus Schollmeier*, Franziska Krause & Uwe Frey, Inst. Neurolol., Neuroreg. & Plasticity, Bremencke- Str. 6, P.O. Box 1860, 38088 Mapleburg, Germany

Hippocampal long-term potentiation (LTP) is thought to serve as an elementary model for the investigation of processes underlying learning and memory formation. Since a possible involvement of GABAergic transmission on the prolonged maintenance of LTP (4 hours) has not yet been shown, we examined the influence of GABAergic antagonists on the maintenance of hippocampal LTP in the CA1 region in vitro. The method used has been described previously by Frey et al. (Brain Res.: 452, 57 - 65, 1988).

Here we present some evidence that bath application of the GABA, antagonist picrotoxin (10 µM) and of the GABA,A antagonist 5-aminovaleic acid (50 µM) did not block the induction and maintenance of LTP at least for 8 hours. Even the application of 50 µM picrotoxin and 50 µM 5-aminovaleic acid together had no influence on the induction or maintenance of LTP. Since application of 10 µM picrotoxin (as used in the first set of experiments) in combination with 50 µM 5-aminovaleic acid did not cause complete inhibition of the GABAergic transmission, 50 µM picrotoxin was used in experiments, where both substances were applied simultaneously.

Our results indicate, that the induction and prolonged maintenance of hippocampal CA1-LTP is independent of GABAergic transmission.

This work was supported by the German BMFT "Nachwuchsgruppen Biotechnologie", FKZ: 0310268A.

623.2

Inhibitory Synaptic Transmission Modulates the Induction of Long-Term Depression in CA1 Region of Hippocampus. P.M. Steele, M.D. Mauth*, Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School-Houston, TX 77225.

The goal of this study was to examine the role of GABAergic input in the induction of long-term depression (LTD) at the Schaffer collateral/commissural synapses in area CA1 of hippocampus slices. The induction of LTD in the hippocampus (Yang et al., Soc. Neurosci. Abstracts, #183, 1993) and possibly in the cerebellum (Elkot et al., Brain Research, 1985) is regulated by GABA. Therefore, we hypothesized that GABAergic input is required for LTD induction in the hippocampus. To test this hypothesis we attempted to induce LTD in the presence of 50 µM picrotoxin (GABA,A antagonist) and again in the same slice one hour after picrotoxin washout. We find that low frequency synaptic stimulation (600 pulses at 1 or 3 Hz) in the presence of picrotoxin produces only a transient decrease of EPSP slope. This decrease returned after 25 minutes to 98% ± 1 of baseline after 1 Hz stimulation and 95% ± 2 after 3 Hz stimulation. In the same slice LTD could be reliably induced after picrotoxin washout; EPSPs were reduced to 86% ± 2 and 80% ± 1 after 1 and 3 Hz stimulation respectively. These results suggest that GABAergic synaptic transmission can influence LTD induction.

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623.3

GABAergic AND DEVELOPMENTAL MODULATION OF HOSONSYNAPTIC LTD AND DEPOTENTIATION IN THE HIPPOCAMPUS. J.J. Wagner* and B.E. Alger, Dept. of Physiology, School of Medicine, University of Maryland, Baltimore, MD 21201.

To test the hypothesis that GABA may regulate the induction of homosynaptic LTD and depotentiation (DPT), we compared the effects of GABA antagonists on hippocampal slices from young (16-22 days) and mature (5-10 weeks) rats. The slices of extracellular CA1 field EPSPs were used to monitor synaptic transmission and spines. Low-frequency stimulation (LFS, 1 Hz, 900 pulses) elicited LTD with respect to baseline synaptic transmission, or DPT from a potentiated level following LTP induction by HFS (100 Hz/1 sec) in young animals, LTD was inhibited by 1 nM CGP 35348 (a GABA,B antagonist), -1.2 ± 2%, n=10 vs a 27 ± 1%, n=9 decrease in EPSPs from control slices. In contrast, homosynaptic DPT was unaffected by CGP 35348. In mature animals, LFS did not induce significant LTD (4 ± 2%, n=38, from baseline), although significant DPT could be consistently elicited by an LFS given 30 minutes after LTD induction (24 ± 5%, of potentiation remaining, n=15). Bicuculline (10 µM, a GABA,B antagonist) had no significant effect on LTD magnitude in young animals, but significantly enhanced LTD expression in slices from mature animals. In addition, after HFS, LTD (relative to the initial baseline) was expressed in mature slices following 3-5 LFS episodes. Our results suggest that the influences of both age, and of prior synaptic activity (i.e. HFS) on LTD induction can be explained by changes in GABAergic systems in young vs mature, and naive vs tetanized slices.

623.4

NITRIC OXIDE REGULATES THE THRESHOLD OF FREQUENCY DEPENDENT PLASTICITY IN AREA CA1 OF RAT HIPPOCAMPUS. P.L. Malenzi* and P.F. Chapman, Graduate Program in Neuroscience and Department of Psychology, University of Minnesota, Minneapolis, MN 55455.

Inhibition of nitric oxide synthase (NOS) blocks the induction of certain forms of long-term potentiation (LTP) and disrupts the acquisition of several different forms of learning. We examined the effects of two nitric oxide (NO) donors and NOS inhibitors on plasticity in area CA1 of the rat hippocampus slice. Standard techniques were used for hippocampal slice preparation. Slices were obtained from Sprague-Dawley rats (14-35 day old) and experiments were performed in a continuous superfusion system. In the presence of an NO donor, stable LTP could be induced by a variety of tetanic stimuli that were normally below threshold for LTD induction. We were able to induce LTD at stimulus parameters as weak as 2 Hz pulses delivered at 10 Hz in the presence of the NOS donor hydroxylamine (200 µM) or 5-aminos- N-acetylpenicillamine (200 µM). In the presence of the NMDA receptor antagonist AP5, a subthreshold stimulus of 25 pulses delivered at 10 Hz in combination with hydroxylamine induced a slowly developing potentiation. The frequency of synaptic potentiation is a critical factor in determining the resulting plasticity. While holding the absolute number of stimulus constant (900), varying the frequency of stimulation from 1 Hz to 30 Hz will shift the resulting plasticity along a curve from depression to potentiation. The results suggest that nitric oxide alters the induction of long-term depression. Supported by a grant awarded to PFC from the Whitaker Foundation.
3.5 Long-term potentiation: Pharmacology II

3.6 Activation of adenosine A<sub>2</sub> receptors potentiates synaptic transmission in the hippocampus
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Northwestern University and Evanston Hospital, Evanston, IL 60201
Adenosine is released as a retrograde messenger following theta frequency stimulation. Adenosine agonists have been shown to decrease synaptic transmission via activation of an A<sub>2</sub> receptor subtype. We have previously shown that non-A<sub>2</sub>-receptor activation significantly potentiates fast-type Ca current in layer II/III pyramidal neurons from the medial entorhinal cortex (Mogil et al., Neuron, 10: 1993). This effect appears to occur via activation of the A<sub>2</sub>-receptor since the effect is observed with the non-selective A<sub>2</sub>-agonist DMPA but is not observed with selective A<sub>2</sub>-agonists. We tested the effect of A<sub>2</sub>-agonists on synaptic transmission in young adult rat transcortical hippocampal slices. Stimulation was applied to Schaeffer pathway and extracellular potentials were recorded in stratum radiatum of CA1. The percentage potentiation was measured under brief stimulation (100 Hz, 1 sec) with a post-tetanic potentiation (PTP) that returned to baseline within 10 min. LTP induced by T<sub>2</sub> in control solution was not affected by subsequent DMPP exposure, however no additional LTP could be induced (although PTP was observed). These results suggest that A<sub>2</sub>-receptors may be involved in the induction of some component of LTP.

3.7 Developmental changes in cellular mechanisms of long-term potentiation: The role of NMDA receptors in the hippocampal maturation of the rat
The role of NMDA receptors in the hippocampal maturation of the rat was investigated. We report that in the immature rat, the NMDA receptor is essential for the generation of long-term potentiation (LTP). This was shown by the observation that in the immature rat, the NMDA receptor is essential for the generation of LTP. In the mature rat, the NMDA receptor is not essential for the generation of LTP.

3.8 Induction of LTP in a sympathetic ganglion needs activation of the 5-HT<sub>3</sub>-receptor
S. Attarzadeh Saboury, S. Appaageorgiou, S. B. Akakpo, & V. V. Hogan* Department of Biology, Texas Southern University* and Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, TX 77004-2515
In the superior cervical ganglion (SCG) of rat, brief preganglionic supramaximal tetanic pulse induced LTP mediated enhancement of the compound action potential in the postganglionic pathway. LTP in the SCG is independent of activation of cholinergic or adrenergic receptors during tetanic stimulation. Serotonin has been reported to present in some of the ganglion interneurons; the small intensely fluorescent (SIF) cells. We examined the role of the serotonin 5-HT<sub>3</sub>-receptor subtype in LTP because it is widely distributed throughout the peripheral nervous system and exists at high density in peripheral neurones. Pretreatment of ganglia with the 5-HT<sub>3</sub>-receptor antagonist MDL22222 (0.5 μM) 1 hr before the high frequency stimulation prevented the expressed LTP. To confirm the role of endogenous serotonin in the generation of LTP, we used ganglia from rats treated with reserpine (3 mg/kg) 24 hrs prior to removal of ganglia. In contrast to ganglia from untreated rats, those from reserpine treated animals showed no compound action potential in the postganglionic pathway but prevented the expression of LTP.

3.9 Histamine and long-term potentiation
R.E. Brown, H.L. Haas, & K.G. Brayman
Long-term potentiation (LTP) of glutamatergic synaptic transmission in the hippocampus, a candidate mechanism for learning and memory formation, is mediated by a number of neurotransmitters. Since histamine can increase calcium influx through the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors, we examined the role of histamine in the generation of LTP, we wanted to see if histamine could also mediate the induction of LTP.
We tested this possibility in the CA1 region of rat hippocampal slices, prepared according to standard protocols. Application of tetanus (10 26.5, 50 Hz) was delivered twice in the same slices with an interval of 140 min. In control experiments this tetanus led to a short-term potentiation (lasting less than one hour) of the field excitatory postsynaptic potentials on both occasions (n = 6, p > 0.05). However, when histamine (100 μM) was washed in 20 minutes prior to the second tetanus, a long-term potentiation was observed which was significant for up to 3 days following the tetanus (n = 8, p < 0.01). A higher concentration of histamine (10 μM) also gave significant potentiation at 60 min following the tetanus but by 90 min the potentiation was no longer significant (n = 7).
We tried to block this effect with the H<sub>1</sub> antagonist, mepramine (1 μM), and the H<sub>2</sub> antagonist, cimetidine (50 μM), applied together before the first tetanus but washout did not block the potentiation. This indicates that histamine did not affect the LTP produced by the combination of weak tetanus and histamine (100 μM) application. In additional experiments we confirmed that histamine could enhance NMDA receptors, we include in this experiment that histamine facilitated the induction of LTP by enhancing NMDA currents.

3.10 Propranolol suppresses LTP induction in both the lateral and medial perforant path inputs to the dentate gyrus.
J.M. Surveys & C.R. Bramham
Dept. of Pharm., Uniformed Services University, Bethesda, MD
Recent evidence suggests that the lateral (LTP) and medial (MPP) perforant path inputs to the dentate gyrus can exhibit different forms of long-lasting synaptic plasticity. Norepinephrine application induces a lasting potentiation of non-adrenergic and non-cholinergic depression of LTP responses, while LTP induction in the LPP, but not MPP, requires opioid receptor activation. β-Adrenergic receptor activation is also known to be important for LTP induction in the dentate gyrus. However, the role of β-receptors in LTP has not been assessed using selective stimulation of LTP and MPP fibers.
High-frequency stimulation (HFS; 100 Hz, 1 or 2 s) applied to the dentate outer or middle molecular layers of the rat hippocampal slices induced selective LTP of LPP or MPP responses, respectively, as assessed by field EPSP slope measurements obtained from the synaptic layers. The perfusate was continuously superfused with a physiological K<sub>P</sub> solution in the granule cell layer. In the MPP, the β-adrenergic receptor antagonist (-)-propranolol (1 μM) reduced in magnitude, but did not completely block, LTP of the field EPSP and population spike. In the LPP, LTP of the field EPSP appeared normal. These results suggest that β-adrenergic receptor activation is required for full LTP induction of both the lateral and medial perforant path inputs to the rat dentate gyrus. Supported by NIH NS23685.
623.11 DELTA AND MU OPIOID RECEPTOR ACTIVATION IS REQUIRED TO INDUCE LTP IN THE LATERAL PERFORANT PATH OF NORMAL, BUT NOT DISINHIBITED, HIPPOCAMPAL SLICES. C.R. Brambati and J.M. Survey. Dept. of Physiology, University of Pennsylvania, Bethesda, MD.

Opioid receptor-dependent LTP has been demonstrated in the lateral perforant path (LTP) input to the dentate gyrus, a system which is thought to use glutamate and opioid peptides as co-transmitters. However, both glutamate receptor pharmacology and the specific action of opioids have not been characterized. High-frequency stimulation (HFS; 100 Hz, 1 s) applied to the dentate outer molecular layer of rat hippocampal slices induces LTP of long-term potentiation (LTP) as assessed by EPSP slope measurements obtained 40 min post-HFS. In slices maintained in normal ACSF, LTP induction was blocked or significantly reduced in magnitude when HFS was applied in the presence of the general opioid receptor antagonist, naloxone (5 μM), the delta receptor antagonist, naltindoline (50 nM), the mu receptor antagonist, CTAP (100 nM), or the NMDA receptor antagonist, AP5 (20 μM), while the kappa-1 opioid receptor antagonist nor-BNI (60 nM) did not affect LTP.

Selective LTD of the LTP was also obtained in slices maintained in ACSF containing picrotoxin (50 μM) which attenuates GABA-A receptor mediated inhibition. However, in disinhibited slices, HFS applied in the presence of naloxone induced LTD equivalent to control values. The results suggest that delta and mu opioid receptors regulate LTP induction in the LPP by a mechanism which depends upon GABAergic inhibition. Supported by NIH NS23865


Physiological functions of the PCOM-derived peptide CLIP (ACTH 18-39) are scarcely documented. Recently, we have demonstrated that an intracerebroventricular administration of CLIP caused a marked enhancement of neuronal excitability in the hippocampal CA1 region as well as a selective increase of paradoxical sleep. The sleep studies led to suggest that the active sequence is located in the N-terminal part of CLIP.

The present study was conducted to test whether CLIP can modulate neuronal transmission in the dentate gyrus and if the N-terminal sequence of CLIP is responsible for these effects. Experiments were performed on freely moving male Wistar rats (8-9 weeks old), housed individually with food and water ad libitum. A monopolar recording and a bipolar stimulation electrode were implanted stereotactically in the granule cell layer of the dentate gyrus and in the perforant path, respectively. For recording of electrically evoked population spikes, an electrode was placed in the M2 region. The sequence was injected into the CA1 region (5 ng/μl, 0.5 μl) to test for its effects on LTP. The amplitude of LTP was found to be significantly enhanced after the injection of CLIP.

623.13 TEMPORAL DIFFERENCES IN THE PHOSPHORYLATION STATE OF PRE- AND POSTSYNAPTIC PKC SUBTYPES DURING LONG-TERM POTENTIATION IN HIPPOCAMPAL SLICES. Koji Fukanaga1, Dominique Muller2 and Eishichi Miyamoto1. 1Department of Pharmacology, Kumamoto University School of Medicine, Kumamoto 860, Japan and 2Center for Medical University, Universit‡it de Genf, Facult‡e de Medicine, CH 1211 Genf 4, Switzerland.

Among the molecular mechanisms that have been proposed to contribute to the induction of LTP in the hippocampus is the activation of Ca2+-calmodulin-dependent protein kinase II (CaMKII) following the stimulation of the NMDA receptor. Recently, we documented long-lasting increases in the Ca2+-independent and total activities of the enzyme as well as an increase in the ratio of Ca2+-independent to total activity following the induction of LTP (J. Biol. Chem. 268, 7853, 1993). It could suggest that autophosphorylation of the enzyme is responsible for the change in CaMKII activity. Here we demonstrate with [32P]-labeled hippocampal slices that high, but not low frequency stimulation applied to two groups of CA1 afferents resulted in increases in autophosphorylation of both α and β subunits of CaMKII I 1 hr after LTP induction. In addition, significant increases in phosphorylation of endogenous CaMKII II substrates, synapsin I and microtubule-associated protein 2 (MAP2), were observed in the same slices. The stimulations of phosphorylation of CaMKII II and its substrates could be blocked by preincubation of slices with an NMDA receptor antagonist, D-2-amino-5-phosphonopentanoate. These results suggest that LTP is associated with increases in phosphorylation of synapsin I and MAP2 during the activation of CaMKII II.


Aluminum (Al) intoxication is known to induce cognitive dysfunctions and multiple forms of neurodegeneration. The precise mechanism of Al toxicity is not clear. In order to investigate the action of Al on synaptic transmission and long-term potentiation (LTP) in area CA1 of hippocampal slices, field potentials were evoked by stimulation of Schaffer collaterals (0.2 Hz) and recorded in the stratum pyramidale of the CA1 region. After stable population spikes were recorded for 30 min in modified ringer solution, Al was applied for 30 min by bath perfusion. Low concentrations (0.68 μg/ml Al) increased the amplitude of the population spike slightly (121%) whereas 2.7 μg/ml (100 μM) decreased the amplitude (75%) and concentrations ≥ 4 μg/ml (150 μM) blocked the population spikes completely. The action on synaptic transmision was reversible. Induction of LTP by high-frequency stimulation in the presence of 0.68 μg/ml Al lead to a reduced level of potentiation compared to control experiments. In the presence of 2.7 μg/ml Al potentiation was further reduced and declined to baseline level within 60 min. Subsequent washout did not lead to any recovery of the signal. For low Al concentrations (0.68 μg/ml), a rebound of the effect was found in case of washout after potentiation. The blockade of the population spike increased to values above those in control experiments (> 250%).

Our data suggest multiple sites of actions of Al on synaptic transmission and LTP. Both stimulating and inhibitory effects were found as a concentration dependent manner. These interactions might contribute to the neurotoxic potency of Al.

623.15 ENDGENOUS SUBSTRATES FOR Ca2+-CALMODULIN-DEPENDENT PROTEIN KINASE II DURING THE INDUCTION OF LONG-TERM POTENTIATION IN THE HIPPOCAMPUS. Kohji Fukanaga1, Dominique Muller2 and Eishichi Miyamoto1. 1Department of Pharmacology, Kumamoto University School of Medicine, Kumamoto 860, Japan and 2Centre for Medical University, Université de Genf, Faculté de Medicine, CH 1211 Genf 4, Switzerland.

Recently, it has been demonstrated that the intracerebroventricular administration of CLIP caused a marked enhancement of neuronal excitability in the hippocampal CA1 region as well as a selective increase of paradoxical sleep. The sleep studies led to suggest that the active sequence was located in the N-terminal part of CLIP.

The present study was conducted to test whether CLIP can modulate neuronal transmission in the dentate gyrus and if the N-terminal sequence of CLIP is responsible for these effects. Experiments were performed on freely moving male Wistar rats (8-9 weeks old), housed individually with food and water ad libitum. A monopolar recording and a bipolar stimulation electrode were implanted stereotactically in the granule cell layer of the dentate gyrus and in the perforant path, respectively. For recording of electrically evoked population spikes, an electrode was placed in the M2 region. CLIP was injected into the CA1 region (5 ng/μl, 0.5 μl) to test for its effects on LTP. The amplitude of LTP was found to be significantly enhanced after the injection of CLIP.


Rb is the regulatory subunit of cyclic AMP-dependent protein kinase (PKA) that is expressed primarily in neurons and therefore might play a role in several types of synaptic plasticity. Mice carrying a null mutation in the gene encoding Rb were produced via homologous recombination in embryonic stem (ES) cells. These results established that a compensatory increase in Rb protein in various regions of the brain. The hippocampal slice was selected to employ specific mouse model. In the CA1 region, the mutants show normal field potentials in response to Schaffer collateral stimulation, normal paired-pulse facilitation, and normal long-term potentiation (LTP), even at three hours post-tetanus. In a protocol that reliably produced long-term depression (LTD), induced by 900 pulses @ 1 Hz in slices from control mice produced only a transient depression in mutant slices. Depression following LTD in the CA3 region was similar in both control and mutant mice. A protocol that normally produced NMDA receptor-dependent LTD of the field potential evoked by population spikes. Long-term potentiation (LTP) was induced by food stimuli (10 s) produced no LTD in mutant mice. Thus, the Rb isoform of PKA is necessary for at least two forms of hippocampal plasticity.
624.2


We have previously demonstrated that prolonged exposure of cultured cortical cells to amphetamine (AMP) increases [H]HT binding in a dose- and time-dependent manner. To examine whether there are similar effects of glucocorticoids on cortical cells, the cells were exposed to corticosterone (COR), dexamethasone (DEX) or dexamethasone (DECO) for 1 to 6 days. Glucocorticoids (COR, DEX) significantly induced increases in [H]HT/intact cell binding in a dose-dependent manner, but mineralocorticoid (DECO) did not. Scatchard analysis revealed that subchronic exposure of COR induced reduction of both Kd and Bmax values of [H]HT/intact cell binding as well as subchronic exposure of AMP.

In addition, AMP-induced increases in [H]HT/intact cell binding was enhanced by co-administration of COR. Considering the findings that cross-sensitization occurs between stimulants and a variety of stresses, it is of interest that glucocorticoid (one of the biological indicators for stresses) affects the [H]HT binding and suggested that NMDA receptor/ion channel complex is involved in the cross-sensitization.

624.4


Focal stimulation of fiber bundles from the hippocampus results in the appearance of an EPSP-IPSP-LHP sequence when recording from the DLSN slice preparation. GABA, acting at GABA_A and GABA_B receptors, mediates the inhibitory postsynaptic potential (IPSP) and the late hyperpolarizing potential (LHP), respectively. In addition to these inhibitory responses, excitatory postsynaptic potentials (EPSPs) mediated by excitatory amino acid receptor activation are also recorded from DLSN neurons. We now report that cocaine acts to inhibit synaptic transmission in the rat DLSN through pre- and post-synaptic mechanisms.

Standard intracellular current-clamp and voltage-clamp recordings were made from neurons in rat brain slices containing the DLSN. Orthodromically-induced synaptic responses were obtained before and during drug superfusion. Superfusion of cocaine (1 µM) produced a membrane hyperpolarization due to an outward current that gradually faded during continuous superfusion. The amplitude of the IPSP and LHP were reduced during the cocaine-induced hyperpolarization even after the membrane potential was returned to its pre-cocaine value. Following the hyperpolarization and during cocaine superfusion, the depression of the LHP and IPSP persisted. Voltage-clamp experiments revealed, moreover, that the amplitude of the late hyperpolarizing current (ILH) was decreased throughout cocaine superfusion. Cocaine did not reduce a hyperpolarization produced by bath application of baclofen. In addition, cocaine was able to reduce the EPSP and LHP without producing a membrane hyperpolarization. Our results suggest that cocaine suppresses synaptic responses in DLSN neurons, not only by changing the membrane potential, but also by producing a persistent increase in membrane conductance and by presynaptically affecting neurotransmission between the hippocampus and the DLSN. Supported by DA-07190.
624.5 PROPOFOL FACILITATES SUBSTANCE P-MEDIATED INHIBITION OF CALCIUM-ACTIVATED K-CONDUCTANCE IN A GUINEA-PIG HIPPPOCAMPUS. S. K. Nathanielsz, Dept. of Anesthesiology & CCM, Univ. of Pittsburgh, Pittsburgh, PA 15261, and VA Medical Center, Pittsburgh, PA 15240.

Free-running agent propofol (2,6-diisopropylphenol), DIPRIVAN® specifically augments substance P (SP)-but not VIP-mediated presynaptic responses of guinea-pig inferior mesenteric ganglion (IMG) neurons. Single-electrode current and voltage clamp studies on isolated perfused tissue demonstrated that perfusion of 100 µM SP had no effect on the resting membrane potential or input resistance of principal ganglion cells, but augmented in a dose-dependent fashion the amplitude and duration of membrane depolarization and inward current responses evoked by exogenous SP applied via pressure microinjection from nearby pipets (Picophoretzer). The increase in SP-evoked inward current responses resulted from propofol-induced inhibition of an outward current component which was also inhibited by 20 µM TEA, but not 1 mM TEA. SP-evoked inward current responses augmented by 20 mM TEA were not further altered by the additional administration of propofol. During the SP response in normal K+ solution, intermittent depolarizing voltage step (20 mV, 5 s) 0.05 Hz-evoked calcium-activated potassium (V_{Ca}K) tail currents were transiently reduced in amplitude. Propofol had no effect on V_{Ca}K tail currents in the absence of SP, but extended the time period during which V_{Ca}K tail currents were reduced following SP application for the duration of the prolonged SP-evoked inward current response. These findings suggest that the increase in SP-evoked inward current response by propofol is the result of an increase in SP-mediated inhibition of outward current, and membrane depolarization in response to SP. This action represents a novel mechanism of anesthetic action of propofol not attributable to the known facilitatory property of this compound on GABA-A receptor/channels complexes.

Supported by UACCF Pittsburgh, VA RAG B, FAER & Sysmex Laboratories.

624.7 EFFECTS OF THIOPENTAL AND PHORBOL ON CAI FIELD POTENTIALS IN THE RAT HIPPOCAMPUS Y. C. Tsai, E. Narimatsu, T. Gerhold, S. Kamath and M. Sokolii, and E. Węgrzynowicz*. Dept of Anesthesiology, Univ of Iowa College of Medicine, Iowa City IA. 52242.

Thiopental (THIO) has been used primarily as an induction agent for the past 60 years. Because of its structural difference, it has been assumed that its site of action differs from that of the inhalational anesthetics in producing the anaesthetic state. We studied the effects of thiopental on the electrotonic potential of the CAI cell group of the rat hippocampal slice and its interaction with phorbol di-acetate (PDA). In previous studies we examined the interaction between halothane and PDA. Rats were anesthetized with ketamine, sodium pentobarbitone and 400µm thick slices of hippocampus prepared and mounted in a bath. The Schaffer pathway was stimulated using a platinum bipolar electrode and field potentials were recorded with glass microelectrodes (resistance 3-8 MΩ). The two negatively potentials N1 and N2 were analyzed for amplitude, and latency. N2 was also analyzed for slope of the onset of the potential. The potentials were recorded following which THIO (400 or 800 µM) was applied for 30 minutes. Recordings were again made and then PDA 0.25, 0.5 or 1.0 µM was applied sequentially and potentials again recorded. THIO application resulted in approximately a 50% decrease in the amplitude of N2. Application of PDA 1.0µM resulted in approximately a 20% recovery of the amplitude of N2. This reversal is less than that with halothane. These results support the concept that the inhalation and intravenous anesthetics, at least to some extent, act at different sites.

624.8 HALOTHANE AND PROPOFOL INCREASE THE COFACTOR SENSITIVITY OF PURIFIED BRAIN PROTEIN KINASE C. H. Hemming, J. A. Aller Adams, M. M. Hoffman, Departments of Anesthesiology and Pharmacology, Cornell University Medical College, NY, 10021.

Protein kinase C (PKC) has been implicated in a target for general anesthetics. The activation of purified brain PKC is stimulated by general anesthetics when assayed with a physiologically relevant lipid bilayer preparation in vitro. Here we report the further biochemical characterization of the stimulatory effects of halothane and propofol on PKC activation. PKC was purified to >98% homogeneity from bovine forebrain and assayed with 0.2 µg/ml histone H1, 2 µM 1-2,5-diacetylgluceraldehyde (DG)20 µM phosphatidylserine (PS)50 µM phosphatidylinositol lipid vesicles, 50 mM HEPES (pH 7.4), 1 mM EDTA, 5 mM CaCl2, 10 mM Mg-acetate, and 100 µM 1-2,5-ATP at 30°C for 30 min. The values are expressed as mean±SD. *p<0.05, **p<0.01 (paired t-test value without anesthesia).

Both halothane and propofol increased the sensitivity of PKC to activation by DG, PS, and Ca2+, without affecting its apparent affinity for the artificial substrate histone H1. These data suggest that general anesthetics may stimulate PKC activity not by mimicking one of its regulators, but by stabilizing its active conformation. Efforts are underway to extend these observations made with purified PKC in vitro to endogenous neuronal PKC and PKC substrates in order to further assess the role of PKC mediated protein phosphorylation in general anesthetic action.

Supported by a FAER/BAC Neuroscience Young Investigator Award and a Cornell Scholar Award in Biomedical Science.

624.4 INTERACTIONS OF APV, Picrotoxin and Phorbol Dl-acetate on Halothane Depressed CAI Potentials in the Hippocampus. E. Narimatsu, Y. C. Tsai, S. Kamath, and M. Sokolii*. Dept. of Anesthesiology, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

The effects of the inhalation anesthetic halothane and its interactions with phorbol di-acetate (PDA) and the NMDA antagonist d-2-amino-5-phosphonopentanoic acid (APV) were studied in CAI layer of hippocampal slices. Halothane was applied to CAI slices using 100 µM ketamine and 400 µm thick slices 400µm thick were cut and mounted in a bath. The Schaffer pathway was stimulated using a platinum bipolar electrode and recorded with glass microelectrodes (resistance 3-8 MΩ).

The two negatively potentials N1 and N2 were analyzed for amplitude, and latency. N2 was also analyzed for slope of the onset of the potential. The potentials were recorded following which THIO (400 or 800 µM) was applied for 30 minutes. Recordings were then made and then PDA 0.25, 0.5 or 1.0 µM was applied sequentially and potentials again recorded. THIO application resulted in approximately a 50% decrease in the amplitude of N2. Application of PDA 1.0µM resulted in approximately a 20% recovery of the amplitude of N2. This reversal is less than that with halothane. These results support the concept that the inhalation and intravenous anesthetics, at least to some extent, act at different sites.

These results show clearly that halothane affects neurotransmission in hippocampus, and indicate a possible mechanism for excitatory neurotransmitter systems. Supported by EFA fellowship(YS), by the VA research service and by research grant NS13815 from NINDS.

624.10 PHARMACOLOGY OF TRANSMITTER RELEASE IN THE DEVELOPING CHICK HEART. D. B. Gray* and C. E. Ellison, Department of Physiology and Neurobiology, Univ. of Connecticut, Storrs, CT 06269.

Potassium-evoked secretion of acetylcholine (ACh) and noradrenaline (NE) from cardiac parasympathetic nerve endings in cultured sympathetic cell somas respectively, is mediated by Ca influx. In this report we have examined the calcium pharmacology of ACh release from cholinergic terminals in medullated axons of embryonic stage 40 and hatching chick hearts. At embryonic day 14, evoked ACh release from cholinergic nerve terminals in area can be inhibited by over 80% with 10 µM nifedipine. This sensitivity to nifedipine is gradually reduced to less than 30% at hatching. The release remaining ACh release at hatching can be blocked by either picrotoxin suggesting a switchover from L type calcium channels to N type channels involved in exocytosis-secretion coupling. Cultures of SI 40 sympathetic ganglia release labeled NE during a high potassium challenge (56 mM), and this evoked release is not sensitive to nifedipine. However, sympathetic neurons with live heart cell cultures do show sensitivity to nifedipine (>80%) suggesting that exposure to target may induce coupling of L type channels to transmitter release at least transiently, in these neurons. The effects of muscle target and developmental age resemble those in other autonomic ganglia (Gray et al., Neuron 7:15, 1992).

Additionally, embryonic secretion of vertebrate autonomic ganglia to nifedipine may have implications for hypertensive pregnant women. Supported by a grant to B. Gray from the Catherine and Patric Weldon Donaghue Foundation for Medical Research.
624.11
RILUZOLE: FREQUENCY-DEPENDENT ACTIONS INHIBITION OF SODIUM CHANNELS AND INHIBITION OF SYNAPTIC TRANSMISSION.
R.A. Bertoluzza*, S.L. Gurney, Ph. Bouruet, and J.C.R. Randell, Phamacy.
Rhone-Poulenc Rorer S.A., Central Research, 94403 Visy-sur-Seine, France.
Riluzole (3-(5-fluoro-2-thienyl)-2-aminobenzoic acid) is an anti-convulsant and neuroprotective agent. Promising results Phase II study in
amyotrophic lateral sclerosis. In cultured cerebellar granule neurons and
NG108-15 hybrid cells, riluzole (0.3-30 μM) induced a 5-30 mV negative
shift of Na* current, but had little effect on Na* current activation. Riluzole slowed the recovery of Na* current from inactivation (h: control = 8-20 ms; r: 30-60 ms). Thus, inhibition of Na* currents was frequency-dependent only at high activation frequencies (≥ 20 Hz). Depolarizing current pulses evoked trains of action potentials at 20-
50 Hz that were slowed by 3 μM riluzole and reduced to a single spike
by 10 μM riluzole. Riluzole has previously been shown to inhibit the release of glutamate in vitro and in vivo. In hippocampal slices, riluzole (1-30 μM) increased the extracellular threshold of the Schaffer collateral→CA1 pathway evaluated as the appearance of an evoked pre-synaptic fiber volley and a post-synaptic glutamate-mediated field potential; and increased the latencies of these responses, but did not reduce the maximal response amplitudes evoked at high stimulus intensities. When trains of 10 stimuli were applied at 20-100 Hz, marked "use-dependence" of the inhibitory effects was observed that was not overcome at increased stimulus
intensities. We conclude that use-dependent Na* current inhibition
underlies riluzole's selective inhibition of high frequency electrical activity.
Reduced pre-synaptic excitability would contribute to the inhibition of
synaptic glutamate release and excitatory neurotransmission. This selective
action of riluzole may allow it to exert anticonvulsant and neuroprotective
actions while resisting lower frequency "normal" synaptic transmission.

625.1
INTRACELLULAR CI- DIMINISHES G-PROTEIN-ACTIVATED K+ CONDUCTANCES IN RAT HIPPOCAMPAL NEURONS. R.A. Lenter*,
We made whole-cell recordings from CA1 cells in rat hippocampal slices with various recording electrode solutions. We found that G-protein-
mediated K+-dependent responses are suppressed when 160 mM KCl is in the intracellular recording solution instead of KCl3SO4 or potassium gluconate (KGluc). When KCl3SO4 was used, outward currents of the GABA(A) agonist, baclofen, were reduced by 60% relative to responses in cells recorded with KCl3SO4 or KGluc electrodes. Additionally, nonselective KCl3SO4 responses in the presence of glutamate antagonists and 
NMDA, which were reliably obtained when using KCl3SO4 electrodes, were small or nonexistent in cells loaded with KCl.
Membrane potential and resting input resistance did not differ among
recorded with the different solutions. When the non-hydratable
GTP analog, GTPγS, was included in a KCl3SO4 electrode, cells were
usually hyperpolarized and input resistances were significantly reduced.
However, cells recorded with KCl electrodes containing GTPγS had
membrane potentials and input resistances that were not significantly
different from control. These data suggest that high [KCl], prevents activation of a K+ conductance by GTPγS.
We conclude that internal Cl- can interfere with the activation of G-
protein-activated K+ conductance. It will be important to determine if the
interference occurs at the channel, the G protein or at some other stop.

625.2
GDP IS REQUIRED FOR ACTIVATION OF A GLYBURIDE, ATP- AND CROMAKALIM-SENSITIVE OUTWARD CURRENT IN RAT HIPPOCAMPAL NEURONS. G. Dromerick* and K. Kreutzler, Anesthesiology Research Dept., McGill University, Montreal, Quebec, H3G 1Y6, Canada.
There is no agreement between different groups working on cromakalim (CROM) effects on hippocampal neurons. For example, according to Pelti and
sensitive K+ current in cultured neurons. However, we found that cromakalim decreases a voltage-dependent outward current - probably a delayed rectifier -
in CA1 neurons in slices (Yarowsky et al, Neuron, 1993,18:979). The present data were obtained with whole-cell recording from CA1 pyramidal neurons in slices. In blue cells, held at +54 ± 3 mV (where currents were not discerned) by brief depolarizing pulses: at +2 ± 30 mV, currents diminished by 30 ± 10 %.
When the standard internal solution contained also 1 mM GDP, there was a significant outward current at +64 mV, and CROM increased outward currents at +4.2 ± 0.9 mV by 99 ± 24 % (n=19). The enhanced outward currents were reduced by CROM washout (n=2 cells) and by 10 μM GLYB (in 4 cells). When six other cells were recorded with electrodes containing both CTP (5 μM) and
GDP (1 mM), there was no net outward current at +54 mV and CROM reduced outward currents (at +0 mV, by 38 ± 11%). We conclude that, like many muscle cells, CA1 neurons have outward current channels that are both ATP- and GLYB-sensitive, and are opened by CROM - and therefore resemble classical
KATP Channels - but can be activated only when cysolic GDP is present.
Supported by the Medical Research Council of Canada.

625.4
MUSCARINE-INDUCED INCREASE OF AN INWARD RECTIFIER K+ CURRENT IS MEDIATED BY GIZ IN ATT-20 CELLS. T. Kosaka, T. Ohtsuka, Y. Kario, S. Nakagawa* and T. Nakajima*, Dept. of Anat. and Cell 1.,... and Dept. of Pharmacol. Univ. of Illinois at Chicago, Chicago, IL 60612, and Institue of Med. Sci., Univ. of Tokyo, Tokyo 113.
Muscarine and somatostatin induce an inward rectifier K+ current in a pituitary tumor cell line (ATT-20), and these effects are mediated by a G protein, Gi or Go. To investigate which subtype(s) of Gi mediates these agonist effects, we constructed PTK-
sensitive mutants of a-subunit cDNAs of Gi1, Gi2, and Gi3, and transfected them stably into ATT-20 cells. PTX ADP-
ribosylates the cysteine residue at the 4th from the carboxyl terminal of Giα or Goα, and uncouples those subunits from receptors. We mutated this cysteine residue of Giα to serine. The function of the transfected Gi was examined after the endogenous Gi and Go of the transfected cells were inhibited by wild type Giα cDNAs (Giz, Giza, or Giza, PTX-sensitive) were transfected into ATT-20 cells. Muscarine (100nM) and somatostatin (500 nM) effects on cell lines were examined with the whole cell clamp. Only in the cell lines into which the mutated (PTX-
sensitive) Giza cDNA was transfected, did the muscarine response become PTX-sensitive, suggesting that GiZ couples to the muscarinic receptors and enhances the activity of the inward rectifier K+ channel. For the somatostatin response, transfaction of any of the mutated Giza cDNAs did not alter the PTX-sensitivity of the response. Supported by NSF grant IUB-9207855.

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625.5 COMPARISON OF THE INCREASE IN POTASSIUM CONDUCTANCE BY GALANIN AND OXY-M (OXOTREMORINE M) IN MUDPUPPY PARASOMATIC NEURONS. J.M. Malaglina2 and R.L. Petersen. Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Galaniand muscarinic agonists hyperpolarize muscimuloparasympathetic neurons by activating a similar inwardly rectifying potassium conductance (GK). The present experiments were done to quantify and compare the increase in GK produced by galanin and the muscarinic agonist Oxy-M. All experiments were done on isolated ganglia associated with the canal ganglia of the mudpuppy, Necturus maculosus. Recordings were made using the perfused patch mode of the whole cell voltage clamp technique on cells bathed in physiological solution containing as electrolyte cations 3 mM Ba2+ and 30 mM KCl, 75 mM KCl, 3.6 CaCl2, 10 Hepes. Both agonists produced a concentration-dependent increase in GK. At low concentrations (10 nM galanin and 10 nM Oxy-M) the increase in GK was additive. In contrast, at high concentrations of either agonist the response faded, the response to subsequent application of the other agonist. The time course of GK activation was very different. The increase in GK by Oxy-M occurred rapidly, relatively transiently constant in the presence of agonist, and reversed quickly following removal of the agonist. The galanin-induced GK developed slowly and then faded. The time course of the fade was consistent whether or not galanin was removed from the bath. Previous experiments demonstrated that aspartic acid (AA) activates an inwardly rectifying GK with a time course similar to the galanin-induced GK. At maximum concentrations of Oxy-M or galanin, the AA-induced GK was obscured. Our results indicate that galanin or Oxy-M activate the same inwardly rectifying GK but that the mechanism of activation may be different. We suggest that the galanin-induced increase in GK may be mediated through a second messenger, possibly AA.

Supported by NIH RO1 NS23978.

625.7 INVESTIGATING THE SITE OF Zn2+ ACTION BY SINGLE AMINO ACID SUBSTITUTION IN A VOLTAGE-GATED K+ CHANNEL. S.J. Gibbons1, G. Talukder1, M.M. Tamkur2, H. Shear2 and N.J. Harrison1. 1Anes and Crit Care Pharm/Phys, U. of Chicago, Chicago, Illinois 60637, 2Mol. Phys. and Biophys., Vanderbilt U. Nashville TN.

Zn2+ modulates the gating of a human cloned voltage-dependent potassium channel (Kv1.3) by binding to a specific site on the extracellular surface of the protein (Harrison et al. 1993 Mol. Pharm., 42). We have employed the techniques of site-directed mutagenesis and transient expression of mutant channels in HEK-293 cells to establish the amino acids which are important for the integrity of the Zn2+ binding site. Single amino acids in the S5-S6 domain ("P region") were chosen for mutation by comparing the amino acid sequence of known Zn2+-sensitive (Kv1.4 and 1.5) and insensitive (Kv1.2 and 2.1) channels. Whole cell recordings were made at 25°C, using intracellular solutions based on K gluconate and continuous extracellular perfusion with HEPS-buffered saline containing 3 mM K+. hKvL.1 channels expressed in HEK 293 cells activated at a significantly more depolarized potential than the same channels expressed in mouse L cells. Modulation by Zn2+ was studied. Our initial approach has been to generate mutants of KvL.1 with similar amino acids in the P region to KvL.3, which is very weakly modulated by Zn2+. Activation gating of KvL.3 (AATSK) is shifted to the right (V3a=+25 mV) compared to the wild-type (V3a=+15 mV) whereas activation of KvL.1 (H465K) is not significantly changed (V3a=+15 mV, n=4). Robust modulation of gating by 200pM Zn2+ is still observed for both channels although the right shift (U) is slightly reduced (w.t.+25.3mV, A455K+20.2mV, H465K+17mV, n=3-9). We are presently examining the affinity of these channels for Zn2+.

SIC is supported by DHH5 training grant # DA07255.

625.9 AN ATP-SENSITIVE K+ CHANNEL IN ISOLATED RAT NECORTICAL NEURONS: ACTIVATION BY GANGLIOSIDES Z-TONG and X-D TANG Dept. of Physiol., First Military Medical Univ., Guangzhou 510515, P.R. China

Our previous studies have indicated that gangliosides (GM) protect cerebral ischaemia by measuring infra-red area, contents of free radical and ECG. The ionic mechanisms were further studied here with patch clamp technique. Single neurons were isolated from Sprague-Dawley rat neonates at 8~18°C. The pipette and bath were all filled with TTX (1µM) and CaCl2 (0.3 mM) contained but glucose-lacking solutions, with potassium concentrations of 5, 5.5, 6 or 7.5 mM. In 7 cell-attached patches, 2~10 µM of monosialo-ganglioside-GM1 (Sigma, approx. 95%) produced a class of single channel currents which durations of the burst openings showed concentration-dependence. The values of the burst times resulted from 2, 5, 10 µM GM1 were 6±2 s (n=3), 21.4±3 s (n=3) and 42 s (n=1), respectively. The long-time burst-like openings would disappear after washing GM1 out. After formation of inside-out recording from the same, cell-attached patch, the channel activities were depressed by either 2 mM of Na2-ATP (Sigma) or 1 µM of glibenclamide (Sigma). The reversal potential was ~ 0 mV (pipette plus 2 µM ATP-sensitive type). In 6 inside-out patches, after applications of 5~10 µM GM1, single channel recorded often became multicellular activities which then recovered to single channel openings. The GM-elicted currents were inhibited by applications of 4 mM ATP or 2 µM glibenclamide, with a reversal potential of ~ 0 mV and a channel conductance of ~ 50 pS.

The results demonstrate that, under the conditions of metabolism inhibition (both without glucose and ATP), GM can activate ATP-sensitive K+ channels in rat neocortex. The mechanism was suggested to involve protein kinase C.

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625.10 PROSTAGLANDIN E2 REGULATES VOLTAGE-GATED POTASSIUM CHANNELS IN NEUROHYPOPHYSEAL NERVE TERMINALS. Lei ZHANG, Edward KARPINSKI and Christina BENSON. Dept. of Physiology, Univ. of Alberta, Edmonton, AB Canada T6G 2H7.

A high concentration of prostaglandin E2 (PGE2) has been demonstrated in the neurohypophysis and may have an important role in hormone release from the posterior pituitary. In the present studies, the whole-cell version of the patch clamp technique was used to investigate the effect of PGE2, on potassium (K+) currents in nerve terminals of isolated nerve fibers permeated from the rat neurohypophysis. PGE2, initially increased the delayed rectifier K+ current (Iq).

The effect of PGE2, on Iq, was transient with a return to control levels after 15 to 20 min. Additionally, 15 to 20 min after PGE2, administration, the transient outward current (Iw) was increased. These effects were concentration dependent, within the concentration range of 50 to 500 µg PGE2. forskolin, 8-bromo cyclic-AMP and dibutyryl cyclic-AMP also significantly increased Iq and simultaneously decreased Iw. These results show that cyclic-AMP modulates K+ channels in nerve terminals, and may mediate some of the effects of PGE2 on K+ channels and, hence, hormone secretion from the neurohypophysis.
625.11
5-HYDROXYTRYPTAMINE MODULATION OF DELAYED-RECTIFIER-TYPE POTASSIUM CHANNELS IN ANTENNAL-LOBE NEURONS OF THE MOTH MANDECA SEKTA. F. Kleppenhorst2, A. R. More,1 and J. G. Hildebrand1. 1ARL Div. of Neurobiol., Univ. of Arizona, Tucson, AZ 85721; 2Dept. of Zool., Univ. of Otago, Dunedin, NZ.

A principal goal of our studies of the olfactory system of the sphinx moth Manduca sexta is to determine the role(s) played by 5-hydroxytryptamine (5HT or serotonin) in the antennal lobe (AL, the principal olfactory centers) of the brain. In Manduca, each of the two ALs is innervated by a single SHT-immunoreactive neuron (J. Neurobiol. 49:451-465, 1997). Synaptic contacts between AL interneurons and the SHT-containing cell occur within the glomerular neuropil of the AL. The majority of these contacts are output synapses from the SHT-containing cell (J. Comp. Neurol. 338:5-16, 1993). In the brain of the adult moth, exogenously applied SHT (100 µM) leads to blunting of neuronal excitability and decreases in SP in AL interneurons (J. Neurosci. Abstr. 18:303, 1992). These effects can be mimicked by application of SHT to AL neurons in primary-cell culture (J. Neurosci. Abstr. 19:126, 1993). Both in vivo and in vitro, treatment with SHT leads to an increase in cell input resistance. Here we report that SHT causes prolonged closure of delayed-rectifier-type K+ channels in these cells. Applied to AL neurons in vitro, in brain slices, or in semi-intact brain preparations, SHT (5-10 µM) decreases channel opening probability and causes reversible reduction of delayed-rectifier K+ current. Single-channel recordings in cell-attached mode suggest that the effects of SHT on K+ channels of these type are mediated via a diffusible second messenger, the identity of which has yet to be established. Closure of delayed-rectifier-type K+ channels could account for the increased duration of action potentials observed in many AL interneurons in response to SHT. [Supported by a grant to ARM from the USA/NSF Cooperative Science Program and by NIH grant AI-32525 to JVM.]

625.12

We studied large-conductance, Ca2+-activated K+ (BK) channels in smooth muscle cells from the basilar artery of the guinea pig using the patch-clamp technique. In inside-out patches, the single channel conductance was 260 pS and in outside-out patches, the apparent dissociation constant for block by tetraethylammonium (TEA) was 280 nM. Our principal goal was to assess the contribution of BK channels to conductance at negative potential in the resting membrane potential. We measured Np, (number of channels in the cell x open channel probability) at physiological potentials by recording single channel currents in a whole-cell configuration, a hybrid technique designated the "outside-out-whole-cell" configuration. With physiological solutions inside and outside, including 1 µM Ca2+ internally, values (mean ± S.D.) of Np were 0.17 ± 0.56 for -40 and -30 mV, respectively. Our results on the single channel properties of this current indicate that, under conditions that simulate in part those expected with active myogenic tone, including a membrane potential of -40 mV and [Ca2+]i=0.1 µM, BK channels could contribute on the order of 10-20 mV to the membrane conductance, a value that compared favorably with the amount of depolarization observed under current clamp following external application of 1 mM TEA.

625.13
TETRANYPE: EFFECTS ON BKCa CHANNELS. P. Winkl1, T. Glaser, and H. Sommermeyer. Institute for Neurobiology, Troponovens GmbH & Co. KG, Berliner Strasse 136, 50637 Cologne, FRG.

The aldilid tetranype (TET) is used in traditional medicine in China because of its analgesic, anti-inflammatory and for its vasodilatory and antipertussive properties. Recently, TET has been used for differentiation of certain subtypes of Ca2+-dependent unitary channels of high conductance (BKCa) in rat neurophysiological nerve terminals and par intercellular media (Wang and Lemos, 1992). In these systems, it has been shown to block only (charybdotoxin) CTX insensitive type II currents, whereas QA's sensitive type I currents remained unaffected. In the present study the effects of TET were characterized in detail in two different cell systems. In the rat glioma cell line C6-BU1, ionomycin stimulated rubidium (Rb+) efflux was found to be sensitive to (charybdotoxin, ibitoxin and tetraethylammonium (TEA), while apamin and 4-aminopyridine (4-AP) were ineffective. This pharmacological profile suggests the existence of BKCa currents in these cells. Since ionomycin stimulated Rb+ efflux was not influenced by TET, BKCa channels in C6-BU1 cells seem to belong to the class I BKCa channel subtype. In GH3 (rat anterior pituitary tumor) cells BKCa currents were characterized using the patch clamp technique. In particular, our results in the study above, external applied CTXs and TEA reversibly blocked single channel currents, whereas 4-AP had no effect. Surprisingly, also TET turned out to inhibit BKCa currents in GH3 cells, suggesting the presence of a channel type not classified so far.

To get insight into the role of TET sensitive BKCa channels in the rat brain, TET effects were characterized in microdissection experiments measuring dopamine release from the N. accumbens. Local administration of TET results in a profound and transient increase of dopamine release, while the metabolites DOPAC and HVA were hardly affected. The latter results indicate that tetranype sensitive processes are involved in the regulation of dopamine release in this brain area.

625.15
ETHANOL ACTIVATES LARGE CONDUCTANCE, Ca2+-ACTIVATED K+ CHANNELS IN NEUROHYPOPHYSIAL TERMINALS. A. M. Deptino, J. B. Lemos and S. N. Tristram. Department of Pharmacology, University of Massachusetts Medical Center, and Worcester Foundation for Experimental Biology, Worcester, MA 01605.

Large conductances, Ca2+-activated K+ (maxi K+) channels are thought to underlie interburst intervals and, thus, control hormone release from neurohypophysial terminals. Since ethanol (EtOH) inhibits the release of vasopressin (VAP) from osmotically sensitive (OT) and non-OT nerves, we examined the effects of EtOH on maxi K+ channel currents from these terminals using patch-clamp techniques. Maxi K+ channels show a reversar potential close to 0 mV in excised, outside-out patches in symmetric 145 mM KCl solutions. They have a unitary conductance of 250 pS and increase activity at more positive potentials and/or when [Ca2+] i is increased at the cytosolic side of the membrane. EtOH (25-100 mM) applied to the cytosolic surface of the patch reversarly increases K+ activity without changing the unitary conductance of the channel. This activation by EtOH may reflect a direct interaction with the channel, possibly altering the EIC50 for Ca2+-activation of I-V, and in conjunction with the previously-reported inhibition of L-type Ca2+ channels, can explain the reduced release of AVP and OTR after EtOH ingestion. Supported by N.I.H. grant AA-08003.

625.16
POTASSIUM CHANNELS IN THE HYPERKALEMIC RESPONSE TO SUCINYLCHOLINE FOLLOWING DENERVATION. J. A. J. Martyn, F. Yanez, D. B. Carr*, Dept. Anesthesia, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114.

Denervation induces a proliferation of nicotinic acetylcholine receptors (nAChRs) at the muscle membrane. This upregulation of nAChRs is associated with supersensitivity to agonists such as succinylcholine (SCh) and is believed to be responsible for the potentially lethal hyperkalemic response to SCh observed in patients with denervation injuries. The present study uses K+ channel blockers, 4-aminopyridine (4-AP) and tetraethylammonium (TEA) to characterize the contributory role of K+ channels in this hyperkalemic response.

Rats underwent bilateral sciatic denervation. After 2 weeks, the plasma K+ levels to SCh (3 mg/kg i.v.) was studied. The rise in plasma K+ Induced by SCh was significantly greater in denervated rats (2.9±0.3 mEq/L, n=6) than in controls (0.7±0.2 mEq/L, n=6). Pretreatment with either 4-AP (3 or 5 mg/kg) or TEA (20 or 40 mg/kg) failed to inhibit the rise in K+, TREA (60 mg/kg) attenuated the hyperkalemia to SCh (1, 2, and 3 mg/kg). Pretreatment with either 4-AP (3 or 5 mg/kg) or TEA (20 or 40 mg/kg) failed to inhibit the rise in K+, TREA (60 mg/kg) attenuated the hyperkalemia to SCh (1, 2, and 3 mg/kg). Pretreatment with either 4-AP (3 or 5 mg/kg) or TEA (20 or 40 mg/kg) failed to inhibit the rise in K+, TREA (60 mg/kg) attenuated the hyperkalemia to SCh (1, 2, and 3 mg/kg). Pretreatment with either 4-AP (3 or 5 mg/kg) or TEA (20 or 40 mg/kg) failed to inhibit the rise in K+, TREA (60 mg/kg) attenuated the hyperkalemia to SCh (1, 2, and 3 mg/kg).
625.17
ACTIN FILAMENTS REGULATE ION CHANNELS IN IDENTIFIED RETINAL NEURONS. Greg Maugure*, Victoria Connaughton, Adriana Frattu, George R. Jackson, and Horacio Cantellof, *Sensory Sciences Center, The University of Texas, Houston, inactivate, and are capable of producing maintained depolarization; they play important roles in the physiology and pathology of neuron function. CAN channels have been studied in neurons following bursts and after Ca$^{2+}$ injection, and in patches isolated from retinal membranes. Although CAN channels have been shown to be modulated by a PKA mechanism, no direct drug effects on neural l_{Na} have been reported.

We studied l_{Na} in bursting neurons of Helix aspersa by measuring the difference in tail currents at E_{Na} following 50 and 500 ms depolarizing pulses to 10 mV. These current tails were identified as being l_{Na} because they had decay time constants of at least 500 ms, could be measured at E_{Na}, were eliminated by Ca$^{2+}$ current blockers, and were unaffected by removing extracellular Cl$^{-}$. Flufenamate and menefenamate have been shown to block l_{Na} in non-neuronal but there are no reported blockers of neural CAN channels. While lower concentrations had no effect on neuronal l_{Na} tails, 500 mM of flufenamate or menefenamate had a biphasic effect, first increasing and then depressing l_{Na} tails. This may represent an effect similar to that seen in non-neuronal cells where these drugs first release Ca$^{2+}$ from intracellular stores and then block CAN channels.

626.20
INTRACELLULAR SODIUM EVOKES A SULFONYLUREA-SENSITIVE POTASSIUM CURRENT IN DOPAMINE NEURONS. S.W. Johnson*, K.-Zhong Shen, R. Alan North and Vincent Sejnowski, Departments of Pharmacology and Neurology, Oregon Health Sciences University, Portland OR 97201, USA.

In Parkinson's disease, dopamine neurons may have reduced ability to synthesize the ATP needed to maintain intracellular Na+ homeostasis. To study the electrophysiological effects of increased intracellular Na$^{+}$, we used whole-cell patch pipettes to dialyze Na$^{+}$ (40 mM) into dopamine neurons in the rat midbrain slice. We found that Na$^{+}$-loading increased membrane conductance and evoked an outward current at -60 mV (205 ± 22 pA; n = 36). About 70% of current evoked by Na$^{+}$-loading was blocked by Ba$^{2+}$ (300 mM), glipizide (IC_{50} = 4.4 mM), tolbutamide (IC_{50} = 20 mM), and glibenclamide (1 mM). This current was mediated by K$^{+}$ because it reversed direction at the expected reversal potential for K$^{+}$ (−98 ± 6 mV, n = 9; 100 mM K$^{+}$ in pipette, 2.5 mM in bath), and was also blocked by extracellular TEA (30 mM) and intracellular Cs$^{+}$ (100 mM in pipette). About 30% of current evoked by Na$^{+}$-loading was not blocked by Ba$^{2+}$; this current was generated by Na$^{+}$/K$^{+}$ ATPase because it was blocked by a reduced extracellular concentration of K$^{+}$ (0.5 mM), and by dihydrobiotin (3 mM). We suggest that the sulfonuurea-sensitive K$^{+}$ current evoked by Na$^{+}$-loading may have a neuroprotective function in Parkinson's disease.
626.3

Single electrode voltage clamp experiments were carried out in axotomized X-organ neurons somata of the crayfish (Procambarus clarkii). The outward currents recorded in response to depolarizing voltage pulses, from a holding potential of ~45 mV, were blocked by [Ca]. These currents increased 80% when [Ca] was increased from 1.5 to 13.5 mM, and the same diminution occurred when [Ca] was reduced by 2 mM to 13.5 mM and the same diminution occurred when [Ca] was reduced by 2% when [Ca] was increased from 1.5 to 13.5 mM. A diminution of the same order was observed when 1 mM TEA or 20 mM charybdotoxin were added to the bath. Apamin, however, had no effect. On the other hand, a 200 pS (in 227 mM KC) K channel was observed in inside-out patch clamp recordings of these neurons. This channel is activated when the internal [Ca] is increased from 1 μM to 50 μM. The presence of this high conductance Ca activated K channel may be important as a burst terminating mechanism in these neurons. Supported by CONACYT 1245-J0903.

626.5

How voltage activated potassium currents (Kv) influence membrane potential behavior in lactotrophs and GH3 cells was addressed by characterizing their kinetic and pharmacological properties using whole cell patch clamp techniques. Exponential fits to the decay of Kv showed that both lactotrophs and GH3 cells have 3 components, a slowly inactivating component (Kv1), a slowly inactivating component (Kv2), and an offset current. Voltage at 50% activation and rate of decay of Kv1 was similar in lactotrophs (5 mV & 150 mV) and GH3 cells (4 mV & 140 mV). Voltage at 50% decay activation was -52 mV in lactotrophs and -41 mV in GH3 cells. Rate of recovery from inactivation of Kv1 fit a single exponential (9%) in lactotrophs, while in the GH3 cells the recovery from inactivation of Kv1 fit a double exponential (0.12s (70%) & 6s (30%)]. In both lactotrophs and GH3 cells, 4-aminopyridine, 300 μM, blocks Kv1 (~60%) and Kv2 (~50%). Dendrotoxin (DXT), 100 nM, was found to block only the Kv1 (41%) component in GH3 cells but had no effect on Kv1 or Kv2 in the lactotrophs even at concentrations of 3000 nM.

These results show that although Kv1 components in both lactotrophs and GH3 cells are similar, their differences are perhaps significant in terms of influencing membrane potential behavior. DXT will be a useful probe to investigate the role of Kv1 in GH3 cell function.

626.7
BIOPHYSICAL AND PHARMACOLOGICAL CHARACTERIZATION OF INWARDLY RECTIFYING K+ CURRENTS IN RAT SPINAL CORD ASTROCYTES. Christopher B. Ransom* and Harold Sontheimer. University of Washington, Seattle, WA 98195.

Astrocytes are known to possess a large resting K+ conductance. We characterized the resting conductance of astrocytes derived from spinal cord and found insulated and conducting was provoced by inwardly rectifying K* (KIR) channels. We identified two types of astrocyte KIR channels with single channel conductances of -28 pS and -60 pS respectively. Channels displayed voltage dependent block: inwardly rectifying KIR channels are present near and negative to the cells' resting membrane potential, but were near zero at potentials positive of the resting potential. The conductance (pK) of inwardly rectifying KIR channels (KIR) depolarized and the pK was approximately proportional to the square-root of the [K+]o. KIR currents inactivated in a time and voltage-dependent manner due to block by Na+. KIR currents were also blocked by a dose-dependent manner by extracellular Ca* (K=72 μM to -160 μM), Ba* (K=8.5 μM) and TEA (90% block at 10 μM), but were insensitive to 4-AP (5 μM), Ba* and TEA inhibition of KIR currents caused a marked depolarization that suggests a Kir channel activity is essential to establish the negative resting potential typical for astrocytes. The biophysical features of astrocytic inwardly rectifying KIR channels are compatible with properties required for their proposed involvement in KIR clearance according to the "gial spatial buffer" hypothesis: (i) high open probability at the resting potential, (ii) increasing conductance with increasing [K+]o, and rectification, e.g. channel closure at positive potentials. It is supposed, therefore, that the diarrpsis of [K+]o following neuronal activity is mediated by the activity of astrocytic KIR channels.

626.8
POTASSIUM CURRENTS UNDERLYING ACTION POTENTIALS OF HIPPOCAMPAL CA1 ST. ORIENS-ALVEUS INHIBITORY NEURONS. L. Zhang* and C. J. Mellan. Laboratory of Cellular and Molecular Neurophysiology, NICHS, NIH, Bethesda, MD 20892.

Whole-cell recordings were made from 88 CA1 st. oriens-alveus interneurons in hippocampal slices from the rat. The amplitude of potassium currents underlying single action potentials (AP). AP repolarization was dependent on the transient D- and A-currents, since the spike width was broadened and 120% by 100 nM 4-AP respectively. Another prepulse to -80 mV to deactivate the A current increased the role of spike repolarization. A role for the Ca2+-activated K current (Ica) in spike repolarization was also determined, the specific blocker benoxotin (IBTX, 100 nM), increased the duration of APs by 36%. Ca2+-free Co2+-containing solution produced similar effects (30% increase) which were non-additive with IBTX. In contrast, 4-AP (2 mM) further prolonged the APs in the Ca2+-free medium by 130%. APs were followed by two prominent AHPs of fast (2 ms) and medium (270 mV) duration. IBTX (100 nM) or Co2-free solution completely eliminated this fast AHP, while 4-AP was without effect. The amplitude of the medium duration AHP was reduced by approx (100 nM Ca2+ 26%), Ca2+-free Co2+ solution (63%), the muscarnic agonist carbachol (10 μM, 20%), and ketamine (10 μM, 25%). A slow inactivating and two transient outward currents could be elicited by membrane depolarizations in excised outside-out, or whole-cell patches. Subtraction of the currents elicited by the pulse to -90 or -40 mV allowed isolation of each current. The half-maximal activation of the total transient current was reached at -22 mV and the slow inactivating at -4 mV. At -10 mV, 4-AP (2 mM) inhibited both the transient current by 66% and the slow-inactivating current by 11%. In conclusion, Ia, and Ica are three of the major K currents involved in AP repolarization; while ICl,K and IG mediated part of the AHP in O-A inhibitory neurons.
626.9
POTASSIUM CURRENTS IN PREVERTEBRAL AND PAR VERTEBRAL SYMPATHETIC NEURONS. Hong-Sheng Wang and David McKinster*, Dept. of Neurobiology and Behavior, State University of New York, Stony Brook, NY 11794-8010.

Intracellular recordings were made from rat sympathetic neurons in isolated superior cervical ganglia (SCG), celiac ganglia (CG), and superior mesenteric ganglia (SMG). Based on their response to a maintained depolarizing current stimulus, neurons were classified as 'phasic' or 'tonic'. All neurons in the SCG were phasic, 86% of the neurons in the SMG and 58% of the neurons in the CG were tonic and the balance were phasic. The voltage response of the tonic neurons became more depolarized during a prolonged current step. The voltage change induced in these neurons was slower than that produced by a constant current step was markedly different. The response of phasic neurons was biphasic with an initial depolarizing response followed by significant repolarization of the membrane potential. In contrast, tonic neurons became more depolarized during a prolonged current step. The underlying currents were studied using single-electrode voltage-clamp recording. The M-current was present in all phasic neurons, but was very weak or absent in tonic neurons. An A-current was apparent in both phasic and tonic neurons. The slow activation of this current contributed to the slow depolarizing ramp seen in response to a maintained depolarizing current step. Computer simulations, based on the voltage-clamp data, suggested that the different firing properties of phasic and tonic neurons could be accounted for by different expression of the M-current and the D2-current.

626.10
A-TYPE POTASSIUM CURRENT (IA) GATING IS ALTERED IN SYMPATHETIC NEURONS FROM HYPERTENSIVE RATS. Water P. Robertson and Geoffrey V. Schulte*, Dept. of Physiology, Tulane University School of Medicine, New Orleans, LA 70112.

Increased sympathetic nerve activity has been implicated in the development and maintenance of hypertension. The cellular basis for the exaggerated sympathetic neuron activity remains undefined. IA may mediate neuronal excitability via modulation of action potential duration, regulation of the inter spike interval during repetitive firing, and modulation of the time course of EPSP decay. We examined IA in the spontaneously hypertensive rat (SHR) to investigate the possibility that alterations in IA may be involved in the hyperexcitability of sympathetic neurons. Our preliminary data shows that half inactivation voltage of IA from SHR (n=5) is ±13 mV hyperpolarized than control Wistar rats (n=6). In the SHR, inactivation at such hyperpolarized potentials could reduce the availability of IA, and thus mediate the increased sympathetic activity in hypertension.

626.11
ROLE OF IA-DEPOL IN FREQUENCY-DEPENDENT SPIKE BROADENING OF 200 CELLS IN APTYSA CALIFORNICA. M. Mat and J. Roestore, Center for Neurobiology, Columbia University, N.Y., 10032. We studied the mechanisms and consequences of frequency-dependent spike broadening in the 200 SCG neurons in the abdominal ganglion of animals. These neurons, which activate the circuit that inhibits respiratory pumping, contain the neuropeptide SCP. When fired at 4 Hz, the falling phase of the action potential potential was greatly prolonged and could last for more than 30 msec. Spike broadening recorded from the somata of the R20 cells was affected and transmitter release from near terminals. We tape-recorded a 7 Hz, 10 sec train of gradually broadening spikes. They were then replayed at a command signals for a 2-electrode voltage clamp that controlled soma potential. The synaptic output of the R20 cell was recorded in this way in a voltage clamped RGC cell in our cell R20 in the same cell was the same as that produced under current clamp. But when only the first (non-broadened) train was played (5 sec at the same (7 Hz) frequency, the synaptic output was reduced by 50 to 70%. Because the release sites are some distance from the soma, the effect of broadening on release is underestimated by this protocol. Using pharmacological and 2-electrode voltage clamp techniques, we isolated voltage-gated Na, ICa L (L and N-type), ICa H, I Ca N, and I C s in the R20 cells. We observed a range of calcium currents and that for traditional A currents (Platfiger et al., 1991). The contributions of different currents to spike broadening were determined by using the gradually broadening action potential train as the command for the clamp. We found that (i) Although the peaks of ICa show 40% inactivation during the spike current, the time integral of ICa during individual spikes increases by 200%, indicating that there is more Ca influx during broadened spikes than during the non-broadened ones. (ii) Surprisingly, I A depol is the major outward current in the non-broadened spikes. Moreover, if we depolarized and rapid cumulative inactivation, which is the major cause of frequency-dependent spike broadening in the R20 cells.

626.12
IONIC CURRENTS INVOLVED WITH AUTOMATICITY IN A CLONAL CELL LINE. H. Bryant, V. Kowtha, K. Kwass, D. Stenger, Center for Bio/Molecular Science and Engineering, Naval Research Laboratory, Washington, DC 20375. Department of Physiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, Section on Biophysics of Sensory Processes, LC 671/CDD/N, Bethesda, MD 20892. NG108-15 (neuroblastoma x glial cells) can display altered spontaneous activity (the spontaneous occurrence of regenerative action potentials) following transient exposure to extracellular perfusates containing 20 mM MgCl2. The electrical activity of NG108-15 cells was monitored using single-electrode voltage-clamp recording and whole cell patch clamp techniques after culture in serum-containing medium (SCM) or serum-free media (SFM) which was insensitive to TEA and cesium. Action potential profiles of SFM cells were characterized by prolonged after hyperpolarization (AHP) which predisposed SFM cells to fire repetitively. Cadmium blocked the AHP. K+ channel blockers Apamin and Charybdotoxin reduced the AHP component while CTX prolonged the action potential duration. SITS, a potent anion transporter, revealed another chloride sensitive outward component that slowed the firing rate. These results suggest that the AHP and the chloride outward currents present in cells cultured in SFM modulate automaticity in NG108-15 cells in SFM cultures.

626.13
A DENDROTOXIN-I SENSITIVE POTASSIUM CONDUCTANCE IN AUDITORY NEURONS OF THE RAT MNTB. H. M. Brew and J. D. Forsythe. SPON Brain Research Association. Dept. of Cell Physiology and Pharmacology, Univ. of Leicester, P.O. Box 138, Leicester, LE1 9HN, UK.

Neurons of the medial nucleus of the trapezoid body (MNTB) are part of the brainstem auditory pathway. The action potentials recorded were located in MNTB neurons. The MNTB neurons possess rapidly activating potassium currents (Forsythe & Barnes-Davies, 1993. Proc. Roy. Soc. B. 251, p145-150) that are thought to contribute to their ability to follow accurately the action potentials in their single giant synaptic input. We used whole-cell patch techniques to characterize one of the conductances underlying these currents in MNTB neurons of guinea pigs. We found that this is a 0.5mM Ca and 2.5mM magnesium was used for voltage clamp recordings. One potassium conductance produced most of the outward current in MNTB neurons between -60mV and -20mV. This current, IG, is blocked by 100mM D-Phenacetin. The I G, was blocked by 1mM DTX-I (DTX-I) and mostly blocked by 100mM a-aminoprinine. I G activated and deactivated rapidly, reaching a peak value in 8 msec. It then decayed with a mean time constant of 0.1sec. spikes were used with a pseudo-intracellular solution. A modified solution containing 1.0mM Ca and 0.5mM Mg produced I G, I Ca N, I Ca L, and I Ca H. Surprisingly, IA depol is the major outward current in the non-broadened spikes. Moreover, if we depolarized and rapid cumulative inactivation, which is the major cause of frequency-dependent spike broadening in the R20 cells.

626.14

Alpha-Dendrotoxin (d-DTX) isolated from the venom of the African Green Mamba (Dendroaspis angusticeps) has previously been reported to be a neurotoxic peptide that selectively labels with high affinity voltage-dependent potassium (K+) channels. Previous studies have extensively studied the binding profile of tritiated d-DTX in rodent and bovine brain. This study compared the radiiodinated binding profile of [125I]-d- DTX in hippocampal tissue of non-human primates as well as human brain tissue using saturation analysis, competition experiments and quantitative receptor autoradiography. A rapid and reliable method was developed for the preparation of [125I]-d- DTX using whole cell agglomerates of brain tissue. The Kd and Bmax determined by saturation analysis from hippocampal tissue of rat, cynomolgus monkey and human were 63 ± 5 pM, 732 ± 91 fmol/mg prot., 81 ± 2 pM, 503 ± 63 fmol/mg prot. and 92 ± 4 pM, 570 ± 27 fmol/mg prot., respectively. Competition experiments showed the displacement of [125I]-d-DTX by peptide toxins which are known to block voltage-dependent K+ channels indicated that the rank order of affinity constants was similar across species: d-DTX > charybdotoxin > mast cell protease I > apamin. This similarity of the pharmacological profile in the hippocampus among the different species was confirmed using a variety of structurally and pharmacologically diverse agents. Interestingly, the relative distribution of d- DTX binding sites in hippocampal tissue as studied by quantitative receptor autoradiography indicated that species differences in localization. Taken together, these data suggest that there is significant conservation of the voltage-dependent K+ channel subunit that binds d-DTX across species but the neuronal anatomical distribution appears to be.
626.16 Cd\textsuperscript{+} BLOCKADE OF HYPERPOLARIZATION-ACTIVATED CURRENT I\textsubscript{L} IN CRYSTALFISH MUSCLE. A. Araque, D. Campan and W. Buls. Instituto Cajal, CSIC, Madrid 28008, Spain. A novel hyperpolarization-activated current mediates inward rectification in opener crystalfish muscle has been recently described (Araque and Buls, J. Neurosci., 1994). This voltage- and time-dependent current, called I\textsubscript{L}, is selectively mediated by Cd\textsuperscript{+}, its activation curve depolarizing conductance is unaffected by K\textsuperscript{+}. It is insensitive to Ca\textsuperscript{2+}, Ba\textsuperscript{2+} and Pb\textsuperscript{2+}, but is blocked by extracellular Zn\textsuperscript{2+} or Cd\textsuperscript{2+}. The effects of Cd\textsuperscript{2+} on I\textsubscript{L} were studied under two-electrode voltage-clamp in opener muscle fibres. Cd\textsuperscript{2+} (10 mM) totally and reversibly reduced I\textsubscript{L} without modifying the I\textsubscript{L} equilibrium potential. Lower Cd\textsuperscript{2+} concentration decreased the maximal conductance of I\textsubscript{L} and shifted its activation curve towards more hyperpolarized potentials without affecting the slope of the activation curve. While the I\textsubscript{L} activation time constant increased, the I\textsubscript{L} deactivation time constant was not modified by Cd\textsuperscript{2+}. The effects of Cd\textsuperscript{2+} on I\textsubscript{L} were independent on K\textsuperscript{+}. Cd\textsuperscript{2+} effects on I\textsubscript{L} were dose-dependent, obeying the Hill equation with n= 0.452 and 0.045 m/s and a Hill coefficient of 1.23.

We conclude that: a) the block of I\textsubscript{L} by Cd\textsuperscript{2+} was exerted in a dose-dependent manner, obeying the Hill equation and suggesting that one Cd\textsuperscript{2+} ion binds reversibly to one receptor with a dissociation constant of 0.452 mM; b) the selective permeability of I\textsubscript{L} channels was not modified by Cd\textsuperscript{2+}; c) the binding site for Cd\textsuperscript{2+} to block I\textsubscript{L} was different to the binding site for K\textsuperscript{+}; d) the Cd\textsuperscript{2+} blockade of I\textsubscript{L} was not voltage-dependent; e) Cd\textsuperscript{2+} blocked the I\textsubscript{L} channel gate interfering with its opening but not with its closing mechanism.

Supported by DGICYT (Spain) and DGXII CEIC (Europe) grants to W.B. A.A. is an Arces Foundation postdoctoral fellow.

626.20 EFFECTS OF INTRACELLULAR PH ON MEMBRANE ELECTRICAL AND MECHANICAL PROPERTIES OF VASCULAR SMOOTH MUSCLE.
Brigette Liu, P.C. Johnson and G.E. Koshland*, Department of Physiology, University of Arizona, Tucson, A285724

We have investigated the effect of changing extracellular pH (pH\textsubscript{e}) at constant level of CO\textsubscript{2} on the membrane electrical and mechanical properties of vascular smooth muscle in isolated guinea-pig mesenteric and femoral arteries, using intracellular microelectrode recording and force measurement. Lowering pH\textsubscript{e}, depolarizes but reduces force development of vascular smooth muscle, whereas increasing pH\textsubscript{e} hyperpolarizes and increases force development. From measurement of the decay time constant of excitatory junction potentials, we also found that lowering pH\textsubscript{e} from 7.4 to 6.6 decreased the total membrane conductance of vascular smooth muscle whereas increasing pH\textsubscript{e}, had the opposite effect. The relationship between membrane conductance and pH\textsubscript{e} was characterized as we increased the [H\textsuperscript{+}], (in mM) from 4.7 to 25 at pH 6.5, 6.7, and 7.8. The value of membrane potential measured at [H\textsuperscript{+}] of 25 mM was close to that predicted by Normax equation. These data indicate that the resting potential of vascular smooth muscle is predominantly determined by the membrane conductance to K\textsuperscript{+} and that the effect of pH\textsubscript{e} on potential may be mediated through altering K conductance. Finally, application of cromakalim and glibenclamide did not significantly affect the resting potential at pH 6.5, 6.7, 7.4, and 7.8, which suggests a lack of effect of pH\textsubscript{e} on K channel activity.

The maximum effect of pH on membrane potential occurs within about 5 min, after which the potential tends to return toward the control level. This secondary change is blocked by 1 mM TEA, suggesting that it is due to change of intracellular pH acting on Ca-regulated K channels. Supported by NHL grant ID. 15790.
627.1 ANALOG VLSI MODEL NEURON: A MULTIPLE PURPOSE PROGRAMMABLE DEVICE. G. Le Masson*, S. Le Masson, Y. Devill, and M. Moulines. LNPs, UN. Bordeaux France. Arcachon 33120 FRANCE and IXURA CNRS 8464 Bordeaux FRANCE.

Using a BCGMs full custom technology, we developed an analog VLSI circuit (ASIC) for modeling conductance-based neurons. Currents with voltage dependent, sigmoidally-shaped activation and inactivation are produced and summed through an external external serial capacitance. The maximal gain and voltage dependence of each current is programmed analogically via analog inputs, as well as the slope of both activation and inactivation curves. Simple kinetics and calcium dependent currents are available, and a synaptic current. With the construction of this circuit, we assembled several circuits is possible, to model neurons with complex intrinsic properties such as burstiness, or to reproduce multiartificial networks. We recently reported a new technique for real time interaction between model neurons and biological neurons, trough artificial synapses. These VLSI models are ideally suited for such hybrid network interactions.


We have examined the possibility of different conditions for the existence of slow oscillations underlying the bursting behaviour (Eckmann, et al., Nature. 362:25,1990; Destexhe et al., Biophysics. J. 65:1388,1993). The problem has been solved by searching for several nonintersecting regions in the parameter space of the membrane (Hodgkin-Huxley) equations (Guckelbusch et al., Phil. Trans. R. Soc. 341:345,1993). Since the considered system has two slow variables: intracellular calcium concentration (Ca) and activation variable for the Ca-current (ZCa), two systems have been chosen for the analysis of the conditions of slow oscillations. The first system contains the equations for the membrane potential V and Ca, the second system involves the equations for V and ZCa. Using the methods of bifurcation theory we have obtained for each system the explicit parametric forms of the boundaries limiting the regions of slow oscillations. Analyzing these forms we have found that over the range of physiological values of the model parameters there are two nonintersecting regions on (gCa,f, (gCa,l) and (gK,Ca,l) planes, where gCa,f, gCa,l, gK,Ca,l are the maximal calcium, calcium-dependent potassium and transient potassium conductances and f is the stimulus value. These results suggest that there are multiple conditions for slow oscillations in the membrane of the stomatogastric ganglion. By means of numerical integration we have tested that the frequency of slow oscillations for the parameter values belonging to the regions obtained for the V, ZCa system is lower than the frequency of oscillations obtained for the V, Ca system. The neuronal membrane is likely to display the "slow bursting mode", which has a slow component determined by intracellular oscillations and a fast component caused by oscillations of the variable ZCa. Supported by the N.N.-Stiftung i.G., Wuerzburg.

627.3 RECONSTRUCTION OF HIPPOCAMPAL CA1 PYRAMIDAL CELL ELECTROPHYSIOLOGY BY COMPUTER SIMULATION. D.M. Durand*, E.N. Warman, and G.L. Yuen, Dept. of Electrical Engineering and Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

Computer models of neural activity have been very successful in simulating the neuropsychological behavior of many neurons. In the hippocampus, the electrophysiology of neurons such as the CA1 cells has been studied in great details. A large number of ionic currents have been described but previous computer models were not able to reproduce several important features of these cells. We have developed a model based on the simplest combination of ionic channels that could best reproduce CA1 electrophysiology. The model was designed using seven active ionic conductances and five passive conductances and whose kinetics have been inferred from the available voltage clamp data. Particular emphasis was placed on accommodation, slow depolarization potential and spike broadening during repetitive firing. The activation potential was newly simulated and the role of the three regaining currents Iiring, Iinter and Idep investigated. The model also reproduces all four after-potentials recorded following activation of the cell. The fast, medium and slow AHPs were generated by Iinter, Irep and Idelta respectively. The model accurately reproduces features observed during the injection of long current pulses such as changes in firing frequency and a gradual broadening of action potential. The role of each current in mediating these responses has been investigated. The model also suggested that Irep may be the principal outward current regulating the CA1 resting potential. Supported by NSF grant # BNS 8809504.

627.4 A COMPUTATIONAL MODEL OF MYELINATED AXONS IN FROG DORSAL ROOT. A. L. Paddick* & T. Hashiguchi. Dept. of Pharmacology & Therapeutics, McGill University, Montreal and Dept. of Physiology, Tokyo Medical College, Tokyo.

We have used a mathematical model to simulate electrophysiological characteristics of large myelinated axons. The model describes the space-clamped, intracellularly recorded membrane potential oscillations of the axons. The model is based on a single cable-like neuron model, which accounts for the ionic currents and the anatomy of the myelinated axon. Oscillations of the myelinated axon were simulated by Hodgkin-Huxley type of equations, including inward rectifier and fast and slow K⁺ conductances. The model allows to obtain all known sections of myelinated axons, such as nodal, internodal, myelin, paranodal sections, sections for periodic short and parallel internodal resistances and their measured or estimated properties.

In order to simulate ETPs the myelin leakage conductance and the ion conductance of internodal membrane were found to be crucial factors. In contrast to the classical estimate of 1.5 μS/cm² a much larger value of 80 μS/cm² is required as specific myelin conductance. The likely pathways for this conductance are Schmidt-Lantermann incisures or paranodal opening. The conductance of internodal axolemma requires values that are <1% of the measured conductance of the node of Ranvier.

The model supports the notion that not only the ion conductances of node of Ranvier but also those of the internodal axolemma contribute to electrophysiological properties and conductance mechanism in myelinated axons. (Supported in part by MIUR of Canada)

627.5 BURSTING, SPIKING, CHAOS, FRACTALS, AND UNIVERSALITY IN BIOLOGICAL RHYTHMS. T.R. Chay*, T.S. Lee and Y-S. Fan. Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

Biological systems offer many interesting examples of oscillations, chaos, and bifurcations. Oscillations in biology arise because most cellular processes contain feedbacks that are appropriate for generating rhythms. These rhythms are essential for regulating cellular function. In this tutorial review, we will discuss an interesting nonlinear dynamical process in biology that give rise to bursting, spiking, chaos, and fractals: endogenous electrical activity of excitatory cells and Ca⁺ release from the Ca⁺ stores of excitable cells induced by hormones and neurotransmitters. We will then show how to utilize bifurcation analyses to gain a deeper insight into the mechanisms involved in the neuronal and cellular oscillations. With the bifurcation diagrams, we explain how spiking can be transformed to bursting via a complex type of dynamic structure when the key parameter in the model varies. We will show that the bifurcating structure is important in predicting and controlling abnormal biological rhythms. Although we describe this type of dynamical biological rhythms, we will show that there is universality in their bifurcation structures.

627.6 MEASUREMENT ERRORS FROM CLAMPING VOLTAGE-DEPENDENT CONDUCTANCES IN CELLS WITH ELONGATE PROCESSES. Daniel K. Hartung* and Ann M. Caspel-Franco. Békésy Lab., Univ. of Hawaii, Honolulu, HI 96822.

Voltage clamp data are often obtained from cells with attached processes. Space-clamp errors potentially invalidate such data. To assess these errors, clamp currents were simulated for somata with a single cylindrical process containing voltage-dependent Hodgkin-Huxley (HH) conductances. Leak-subtracted (I) and (IV) data were fitted with HH parameters as if the cells were space clamped. Time constants (τs) for 50% (as opposed to mHs) were voltage-independent. Resulting clamp currents had an activation delay and voltage-dependent kinetic fit broadening the range of parameters by an mH form. For an outward current mechanism uniformly distributed in a compact (0.5%) space, activation τs were lengthened up to several fold, with less effect on inactivation τ. Errors in activation τ and q increased, and in inactivation τ decreased, with increased conductance densities. Fitted kinetic parameters for both activation and inactivation displayed a space-clamp voltage dependence near threshold.

Parameters of the (IV) curve could be well fitted up to ca 10-100x leak conductance density. Measurement errors depended strongly on the time of the measurement, well after peak current being optimal. For a uniformly distributed conductance, measurement error was most severe in conductance regions and away from the peak current activation midpoint and reversal potential. If enough is known about the distribution of channels in processes, it may be possible to correct clamp data for some of the measurement errors.
**627.7**

ENTORHINAL CORTEX LAYER III NEURONS: CHARACTERIZATION AND CHOLINERGIC EXCITATION. A. Menas* and A. Alonso, Montreal Neurological Institute and McGill University, Montreal, QC, CANADA H3A 2B4

Layer II and III of the entorhinal cortex (EC), via the perforant path, project massively to the hippocampus, and receive input from the cholinergic neurons of the basal forebrain. By means of intracellular recordings in the in vivo slice preparation, we have already characterized the electrophysiology of neurons in layer II (Alonso and Klink, 1993) and studied the modulation of their excitability by carbachol (CCh) (Alonso and Klink, 1991 and this meeting). Here, we report on the extension of this study to layer III neurons. Layer III cells appear a rather homogenous population both morphologically and electrophysiologically. Intracellular staining with biocytin revealed a typical pyramidal shape with a thick apical dendrite and a skirt of basal dendrites. Current clamp recordings showed prominent fast inward rectification in both the depolarizing and hyperpolarizing directions. Many neurons displayed dynamic tonic firing at rest which was rapidly maintained by a persistent Na-current. In contrast to layer II neurons, no rhythmic subthreshold oscillations were observed upon DC depolarization. Pressure-pulse applications of CCh resulted in a dose-dependent depolarization which could be potentiated after eliciting firing by manual injection of current. Clamping back to the control level showed an increase in input resistance. With continued bath application (20–60 μM of CCh, the response displayed pronounced desensitization. These data demonstrate clear-cut differences between EC layer II and layer III neurons both in their basic electrophysiological properties and in their modulation by basal forebrain cholinergic input.

**627.9**

RELIABILITY OF SPIKE INITIATION IN NEOCORTEX. Z.F. Mainen* and T.J. Sejnowski, Howard Hughes Medical Institute, Salk Institute, La Jolla, 92037.

Chemical and electrical signalling in the central nervous system appear remarkably variable, yet it is not known whether this variability carries meaningful information or simply reflects the intrinsic unreliability of underlying mechanisms. We have assessed the reliability of one process critical to neural signalling, the generation of action potentials. Whole-cell recordings were made from rat neocortical pyramidal cells in vitro and spikes were elicited by somatic current injection in the presence of synaptic transmission blockers (DNQX, AP5, BMI). Show below are two examples of 10 consecutive responses (superimposed) to repetitions of identical stimulus (scale bars: 50 msec, 0.5 nA, 50 mV). A constant current step (0.2 nA) produced spike trains (f=11–12 Hz) with individual spike times that drifted from trial to trial (top). In contrast, repetition of a stimulus with transients resembling synaptic activity (filtered gaussian white noise, μ=0.2 nA, σ=0.05 nA evoked trains (f=12 Hz) with highly consistent spike times (bottom). Our experiments suggest that some cortical cells are sensitive to the random variability of the input signal which could be through the slow component of Na-current, but the specific mechanism is not clear.

**627.11**

THE HYPERPOLARIZATION-ACTIVATED CATION CURRENT (Ih) IN NEOCORTICAL NEURONS IS BLOCKED BY EXTERNAL PROTEOLYSIS AND INTERNAL TEA. T. Budke*, J.A. White and A.B. Kay, Dept. of Biological Sciences, Univ. of Iowa, Iowa City, IA 52242

After one day in culture neurons derived from the neonatal cerebral cortex exhibited a slowly activating current gated by hyperpolarizing voltage-clamp pulses. The current was blocked by extracellular cesium (2 mM) and unaffected by barium (1 mM) and was permeable to both Na and K (PNa/PK = 0.29). Its form and pharmacology are consistent with a current termed Ih in other preparations. Ih was absent from cells acutely dissociated from both the neonatal and mature cerebral cortex, despite the use of low enzyme concentrations. The sensitivity of Ih to extracellular proteolysis was demonstrated by superfusing the cells with trypan blue (1 mg/ml) while monitoring the presence of Ih in the whole-cell mode of recording. Ih was rapidly abolished (1/2t1/2 ~ 5 min) by proteolysis and exhibited no shifts in its range of activation or changes in its activation kinetics during the course of the digestion, suggesting the abolition of the ability of the current to gate rather than a shift in its range of activation.

Intracellular TEA, at a concentration of ~15 mM was shown to block Ih while extracellular TEA and 4AP had no effect on the current. This suggests that Ih may be structurally related to potassium channels.

Supported by grants from NH and ONR.

**627.8**

WHY DO CORTICAL NEURONS SPIKE IRREGULARLY? M. Todorov, A. Bell, Z.F. Mainen and T.J. Sejnowski,* Howard Hughes Medical Institute, Salk Institute, La Jolla, 92037

The spike trains of many cortical neurons in vivo are highly irregular during both spontaneous and driven activity. Although this variability may simply reflect large correlated fluctuations in synaptic input, an additional consideration is the balance between synaptic drive and the intrinsic conductances generating action potentials.

Using a single-compartment Hodgkin-Huxley model, we show that when excitation and inhibition are balanced to maintain the neuron in the region of optimal sensitivity, near firing threshold, then variable spike trains result even in the absence of large input fluctuations. Increased levels of balanced synaptic input increase the rate of input fluctuations, producing higher firing rates with maintained irregularity. Decreased spike repolarization (weaker K+ rectification) increased the steepness of the input-firing curve, giving greater sensitivity to input fluctuations.

Very similar results were obtained with a simpler integrate-and-fire model, which was used to explore networks of interacting excitatory and inhibitory neurons. Despite positive feedback between the excitatory neurons, proper balance was achieved robustly through inhibitory feedback. With weak repolarization, the network was sensitive to small inputs and could be switched rapidly between stable states dominated by fluctuating membrane conductances.

We conclude that our understanding of K+ conductances (weakening or strengthening spike repolarization) may dynamically regulate the rate of time-scale of information to which cortical networks are sensitive. (We are grateful to M. Shadlen for useful discussion. Supported by ONR. ZFM is an HHMI Predoctoral Fellow.)

**627.10**

Electrophysiological Characterization of Cryopreserved Rat Cortical Neurons T. Wieser, J. Saburin*, M. Wierich*, Boehringer Ingelheim KG, Ingeheim, Germany; *Battelle Europe, Geneva, Switzerland. Primary cultures from embryonic rodent brain are widely used for neurophysiological investigations. Unfortunately, these cultures have only a limited survival time of a few days to weeks, and longer storage is far beyond our reach. We therefore set out to improve the cryopreservation technique of cortical neurons from embryonic rat brain. Cryopreserved neurons were tested using the patch-clamp technique, and their properties regarding voltage- and transmitter-activated ion channels were compared with those from unprocessed controls.

All preserved cells had resting membrane potentials in the physiological range (~55 ±2.8 mV, S.E.M., n=14) and responded to voltage jumps with typical neuronal sodium- and potassium currents. The application of glutamate, NMDA or GABA induced currents indistinguishable from those of the control cells. The NMDA induced currents were suppressed by AP5, the K+ currents by blockers where inhibited by bicuculline and amplified by the steroid 3-alpha-dihydropregosterone.

We conclude that cryopreservation does not alter the properties of cortical rat neurons, and that these cells can be used for electrophysiological and neuropharmacological investigations. Supported by the BMFT (Grant No. 019192A0).

**627.12**

FUNCTIONAL ROLE OF AXON HILLOCK AND INITIAL SEGMENT IN CORTICAL SPIKE INITIATION. J.B. Huguenard* and J. Teyler. Z.F. Mainen and T.J. Sejnowski, Howard Hughes Medical Institute, Salk Inst., La Jolla, CA 92037 and Dept. of Neurosciences, Stanford Univ. Sch. Med., Stanford, CA 94305.

The processes that lead from synaptic input to spike output are critical factors in information processing in cortical neurons, yet the precise mechanisms of spike initiation are not well understood. Although neocortical pyramidal cells have voltage-dependent somatic and dendritic Na+ channels that could promote dendritic spikes, recent data (Stuart and Sakmann, Nature 367:65) support the long-standing hypothesis that spikes are initiated preferentially in the axon hillock or initial segment (AH/IS). This is consistent with our findings in acutely isolated neurons that only cells retaining a visually detectable axon segment can support robust spike generation. A biosynthetic model was constructed in order to explore the implications of these findings. When reported somatic and dendritic Na+ 'dendrites' (40 pS/μm^2) are combined with AH/IS densities at least 200-fold higher, a density comparable to measurements at nodes of Ranvier, spikes are initiated in the initial segment and then invade the soma and dendritic tree. The IS is a lower initiation threshold by a combination of high local current density and electrical coupling to the soma, while the conical shape of the AH promotes complete invasion of spikes into the soma. Simulations of axo-axonic inhibition, as believed to occur at chandelier cell synapses on the AH/IS, demonstrate that this type of inhibition is more effective in delaying spike onset than equivalent inhibition in the somatodendritic compartment. Voltage-clamp simulations indicate that caution should be exercised in the interpretation of somatic voltage-clamp data. For example, IS spike initiation can occur after distal dendritic synaptic input, even with apparent voltage clamp in the soma. These results underscore the capacity of the AH/IS to function independently of the soma, and indicate the importance of further studies of its physiology. Supported by NIH grants NS12151 and Office of Naval Research. ZFM is an HHMI Predoctoral Fellow.
CAESIUM PREVENTS THE MAINTENANCE OF LONG TERM DEPRESSION IN RAT HIPPOCAMPAL CA1 NEURONS. D. DiFrancesco, D. Janigro*, E. Waeke* and G. Macafee. Dip. di Fisiologia e Biochimica Generali, Univ. di Milano, Italy and *Dept of Neurosurgery, Univ. of Washington, Seattle, WA.

Hippocampal synaptic plasticity involves long-term modification of the efficiency of information processing at synaptic sites. We measured long-term depression (LTD) of field EPSP in the CA1 region of spontaneously isolated hippocampal slices by delivering a 15 minutes train of pulses at 1 Hz to the Schaffer-commissural-CAL pathway. LTD was prevented by adding a NMDA receptor antagonist (AP-5, 50 μM) to the perfusing medium. Superfusion of the slices with Cs (2 mM) during or just after the 1 Hz stimulation period could both inhibit the maintenance phase of the depression itself and elicit spontaneous oscillatory activity. Since a major effect of Cs is a block of the hyperpolarization-activated current (Ih, J. Neurophysiol. 69, 2725-2736), these results suggest the possible involvement of Ih in the maintenance of long-term depression and in the regulation of hippocampal excitability.

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Sharp-electrode in vitro microelectrode recordings were made from 43 CA3 pyramidal neurons in vitro, in hippocampal slices obtained from Sprague-Dawley rat pups taken 2-7 days postnatally (P2-P7). Membrane potential oscillations (MPOs) were recorded at resting membrane potential (RMP) or following depolarization to levels more positive than -60 mV, in 31/43 neurons (72%). The RMP of oscillating neurons (+64 ± 1.25 mV, n = 31) did not differ from that of non-oscillating (+63.9 ± 1.38 mV, n = 12). MPO amplitude (3 - 5 mV measured peak to peak at ±50 mV) was about 5-13 Hz, measured at ±50 mV from the RMP and increased upon depolarization. MPOs induced action potential firing, whose frequency increased with depolarization. The percentage of oscillating neurons increased with maturation but not significantly (P2-P10: 15/20 neurons, 65%; P11-P17: 18/22 neurons, 82%). No developmental changes in MPO amplitude or frequency were seen. Intracellular TTX, (1 mM) blocked MPOs in 4/4 slices (P10-P16). Tetraethylammonium (TEA, 5mM), added after TTX, revealed slower, higher amplitude MPOs (2/3 slices) that were subsequently blocked by cobalt (5 mM). Following IPSPs or afterhyperpolarizations, MPOs were transiently inhibited, an effect lasting the return to RMP and not mimicked by negative current injection. The non-NMDA receptor antagonist CNQX (10 μM, n = 2) reduced substantially intrinsic MPO amplitude, and adenosine (50-100 μM, n = 2) did not depress, but increased transiently during washout of MPO amplitude. These experiments indicate that intrinsic MPOs, possibly facilitating synchronization, are present in the majority of CA3 hippocampal pyramidal neurons from the early postnatal life. These MPOs are generated by the activation of Na conductance(s) and possibly Ca-conductance(s) and are depressed by the activation of K-conductance(s).


Neurotensin (NT) excites ventral telencephalic area (VTA) dopaminergic neurons in culture by inducing a nonselective cationic conductance. NT also decreases the inwardly rectifying K conductance induced by the K, aminergic agonists. The I-V relation of the NT-induced current had almost a zero slope between -60 mV and -140 mV. After decreasing the external Ca concentration, the NT-induced current increased by 1/4 times and had a positive slope conductance. This result suggests that in the presence of Ca, the channel blocked by Ca, K, and Cl, are replaced by Ca, and E Cl = -68 mV and E Ca = -13 mV, the nonselective conductance decreases by about 1/4 times, and each between E Ca and E Cl. When the internal Cl concentration was reduced, shifting E Ca from near 0 mV to about -80 mV, the nonselective conductance still decreased by about 1/4 th, suggesting that the nonselective conductance is not incompatible to Cl, and that it was probably a nonselective cation conductance. Neurokinin S (NKB) and ACPD also induce inward currents in VTA neurons. The I-V relations of the NKB- and ACPD-induced currents had almost a zero slope between -60 mV and -140 mV, similar to the NT-induced current. Some of the NKB- and ACPD-responding neurons were found to be dopaminergic based on tyrosine-hydroxylase immunoreactivity.

WHOLE-CELL AND SINGLE-CHANNEL PROPERTIES OF A LINEAR MEMBRANE CONDUCTANCE IN CAT RBCGs. D.W. Robinson*, S.J. Huang, R.P. Scokey and L.M. Chalupa. Dept. of Neurology, Section of Neurobiology, Physiology and Behavior and Center for Neuroscience, University of California, Davis, CA 95616.

Whole-cell recordings from isolated cat reticulocytoglobin cells (RBCGs) revealed the presence of a previously unreported linear current, which increased with ontogeny to a mean conductance of 0.27pS/pF at postnatal ages (Robinson et al., 1993). Changing the external Cl concentration had no measurable effect on the reversal potential or the conductance change that may have been the K conductance. The mean external K concentration was increased from 5 to 35 mM the reversal potential changed from -54 to -18 mV. This change was close to the expected value of 34.5 mV calculated from the GHK voltage equation, assuming a 4% permeability for Na. We also used the cell-attached and flipped-off patch variations of the patch-clamp technique to examine the single-channel basis of this linear whole-cell conductance. From -100 to -100 mV little or no voltage-dependence was observed in the isolated single-channel conductance. Changing K concentrations on both sides of the patch resulted in changes in reversal potential similar to those observed in whole-cell. This potassium channel could therefore play an important role in setting the resting membrane potential in these neurons. Supported by UCD gift 07427 to RPS, NEI gift ET-03991 to LMC and DWR is a HSFS Fellow.

TTX-RESISTANT PERSISTENT NA+ CURRENT UNDERLIES PACEMAKER POTENTIALS OF FISH GONADOTROPIN-RELEASING HORMONE (GnRH) NEURONS. Y. Okai. Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113, Japan.

Gonadotropin-releasing hormone (GnRH)-immunoreactive terminal nerve (TN) cells show endogenous regular beating discharges, which may be related to their putative neuromodulator functions. The ionic mechanism underlying the pacemaker potentials (PPs) was studied using intracellular and patch-clamp current clamp recordings from whole brain in vitro preparation of a small fish brain. Addition of 1.5-3 μM TTX to the Krebs-Ringer solution blocked spontaneous action potentials, but small regular PPs remained. The PPs were not affected by 1 mM amiloride or 1 mM Ni2+ (blockers of low-voltage-activated Ca2+ currents), or 2 mM Co2+ or 0.5 mM Cd2+ ( blockers of high-voltage-activated Ca2+ currents), or in Ca2+-free solution. Thus, Ca2+ currents are not essential for the generation of PPs. On the contrary, the PPs were readily blocked by substituting tetramethylammonium (TMA) or choline for Na* in the perfusing solution, and the resting membrane potential became more hyperpolarized than the control level. This is probably because of the block of persistent inward current that is carried by Na* and supplies the depolarizing drive during the normal beating discharge mode. The present results suggest that the TTX-resistant persistent Na* current, I(Vslo), plays an important role in the generation of PPs in TN-GnRH cells.
628.1 EFFECT OF HYPOTHERMIA AND EXTRACELLULAR PH ON CORTICAL NMDA RECEPTOR ACTIVITY. A.T. Gray, L.T. Buck, J.R. Feinler, B. Hansen and P.E. Richter*. Dept. of Anesthesiology, University of California at San Francisco, S.F., CA 94143-0542

Extracellular acidity is known to inhibit N-methyl-D-aspartate (NMDA) receptor activity and may therefore protect neurons during cerebral ischemia. However, the importance of this effect during induced hypothermia is not clear. Cerebral slices from rats age 10 to 11 weeks were loaded with fluoro-2 and measured cytosolic free calcium. Baseline intracellular calcium was not different between the normothermic (37°C) and hypothermic (17°C) groups (142±8 vs. 102±12 nM, respectively; mean ± S.E.M., n=8 in each group). Baseline ATP levels were 61±11 mmol/mg at 37°C (n=3). Hypothermia reduced NMDA stimulated calcium influx (156±25 vs. 41±6 nM, respectively, n=8 in each group). Extracellular acidity at 37°C decreased NMDA stimulated calcium influx 86% over the in vivo pH range of 6.9 to 7.4. However, no significant effect of extracellular pH on NMDA receptor activity depends on temperature. Further studies are needed to estimate these effects over similar in vivo pH ranges. Supported by the U.CSF Anesthesiology Research Foundation.


During hypoxic brain injury, extracellular pH may drop to as low as 6.4. Most studies to date evaluating neuronal response do so at physiological pH. 7.4. Little is understood about the role of [H+] on promotion/reduction of neuronal death itself.

The role of excitatory amino acid (EAA)-induced phosphatidylinositol (PI) metabolism in neuronal injury is yet to be established. It is now well known, that specifically that stimulate the metabolotropic receptor can cause neuronal injury. In the present study, we investigated the effect of reduced or acidic pH on basal and EAA-stimulated PI metabolism in neuronal cultures. Basal and glutamate-stimulated PI metabolism were decreased at all concentrations tested (10-100 μM), with maximal inhibition detected at 100 μM, when pH was reduced from 7.4 to 6.4. However, during the pH 6.4 reduced both 100 μM glutamate and glutamate stimulation by approximately 25%, stimulation by NMMA was reduced by 77%. Preliminary results indicate that PI metabolism at pH 6.4 is slightly reduced for the non-EAA agonist norepinephrine while unaffected for carbachol. Our results indicate that PI metabolism, via direct (trans-ACPD) or indirect (NMMA) metabotropic receptor activation, is decreased by acids and are consistent with the known effects of pH on NMMA receptor function. Acidic pH effects on multiple EAA receptor types may be beneficial to neuronal outcome subsequent to CNS trauma.

628.3 VISUAL TOXICITY OF ETHAMBUTOL IS MEDIATED THROUGH THE NMDA RECEPTOR OF MAMMALIAN RETINAL GANGLION CELLS. J. L. Lee, L. E. Moscatelli, and E. B. Dever. Harvard Medical School, Dept. of Ophthalmology, MEEL, Dept. of Neurology, Children's Hospital, Boston, MA, 02115.

Ethambutol is a mainstay in the management of tuberculosis and other mycobacterial infections. Although its ability to cause optic neuritis and visual loss is well known, a mechanism has not been identified. We have established that ethambutol is directly toxic to retinal ganglion cells in vitro, and that this toxicity is mediated through the NMDA receptor. In cell culture studies we have shown that ethambutol at 0.1-10 μM prevented cell death in cultures results in the death of 50% of retinal ganglion cells within 24 hours. Ethambutol mediated retinal ganglion cell death is dose-dependent. Other cell types in these preparations are unaffected. This toxicity can be prevented in several ways: (1) the elimination of endogenous glutamate; (2) the addition of the NMDA antagonists APV (2-aminophosphonovoronic acid) or the calcium blocker nimodipine. In addition, the non-NMDA antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) did not reduce toxicity. Taken together, these experiments suggest: (1) ethambutol is directly toxic to retinal ganglion cells in culture; (2) ethambutol increases the sensitivity of retinal ganglion cells to endogenous levels of glutamate; (3) ethambutol toxicity is mediated through the NMDA receptor. These findings suggest that visual loss due to prolonged ethambutol treatment for tuberculosis may be prevented by the use of selective NMDA antagonists.


The pathologic role of cAMP in excitotoxic brain injury was assessed in a perinatal rat model. Stereotaxic intrastriatal injections of 8-bromo-cAMP or dibutyryl-cAMP (600-1200 nmol, 0.3 μl) did not cause cAMP analogues of vehicle, produced no apparent permanent or behavioral brain injury in PND 7 rats. The severity of brain injury was assessed histologically and by hemispheric weight disparities 5 days following injection (Mcdonald et al., 1989).

The cAMP analogues were administered as a monotherapy or in combination with the NMDA antagonist MK-801 (2.5 mg/kg, i.p.). The combination of cAMP analogues and MK-801 provided significant protection of MK-801 alone, suggesting that the cAMP analogues activate the cAMP cascade which is inactivated by the NMDA antagonist.


Glutamate activates two broad categories of receptor subtypes, ionotropic (i.e., selective agonists AMPA, Kainate, or NMDA) and metabotropic (mGluR). Maintaining the balance between plasticity and pathology may involve receptor subtype-specific and neuron-specific countermeasures including the glutamatergic antagonist dihydroxy-cyclopentenol-1,3-dicarboxylic acid (DCAC), a selective mGluR agonist, to attenuate NMDA neurotoxicity (Kob et al., 1991) in cortical cultures. Although other neuroprotective actions of ACVD have been demonstrated both in vitro and in vitro, recent studies indicate neuroprotective effects of mGluR activation with relatively low doses of ACVD. In the present study, we examine the interplay between NMDA and ACVD to determine if the neuroprotective or neuropathological effects of mGluR activation.

Fifteen adult male Sprague-Dawley rats were treated with intrahippocampal injections of 10 mM ACPD (100 mM NMMA, and in the contralateral side 10 mM ACPD or 100 mM NMMA. At 14 days after the injections, ACPD resulted in minor damage to the dorsal blade of the dentate gyrus, whereas NMMA alone did not produce significant neural damage. The combination of ACPD and NMMA produced significant damage to CA3 and also involved the ventral blade of the dentate. Excitotoxic actions of the ACPD+NMMA may indirectly modify spine or directly effect receptors. The potential neuroprotective and toxic activity of mGluRs may be an important factor in normal CNS development, disease-related pathology and provide insights into the development of therapeutic interventions.

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NDMA TOXICITY IN DEVELOPING CEREBELLAR GRANULE CELLS MEDITATED BY A MANGANESE-SENSITIVE NDMA RECEPTOR. T. Xi, K.R. Yan, J. Ban, B. Michaelis, and R. Michaelis. Pharmaco. & Toxicol. Dept., Univ. of Kansa, Lawrence, KS.

NDMA receptors play an important role in glutamine-induced neuronal toxicity and are related to neurodegenerative disease. Cerebellar granule cells in culture were used as a model to study the role of NDMA-mediated neurotoxicity. Rat cerebellar granule cells were grown under depolarizing conditions for 7 days, and the medium was then changed except for the addition of D-glutamate to compensate for evaporation. The cells were treated with 50 µM NDMA that was directly added to the culture medium in the presence of 0.8 µM Mg²⁺.

Cell death was monitored by both the LDH and the MTT assay. The dose response curve of NDMA-induced toxicity showed that the toxicity was increased through DIV 8 to DIV 14. This increased toxicity correlated to the increased expression of the 63-70 kDa glutamate-binding protein as determined by Western blot. The NDMA (100 µM)-induced toxicity at DIV 14 was blocked by AP-5 (500 µM) and NMDA (10 µM) and it was not sensitive to CNQX (25 µM). This indicates that the NDMA-induced neurotoxicity is mediated by NMDA receptors rather than non-NMDA receptors which might have been activated as a result of NDMA-induced glutamate release. The NDMA-activated increase in [Ca²⁺], measured by Fura-2 in the condition favoring the Mg²⁺-sensitive NMDA receptors (5 nM KCl, 10 µM glycine, and Mg²⁺-free) did not show the developmental increase through DIV 5 to DIV 14 and did not correspond to the developmental pattern of NDMA-induced toxicity. These results may suggest a distinctive role of Mg²⁺-insensitive NMDA receptors in NDMA toxicity of cerebellar granule cells.

Supported by a Marion Morrison Dow Fellowship and a Grant.


Memantine is a potent noncompetitive antagonist at N-methyl-D-aspartate-activated receptor channels (J. Pharmacol., 166, 591, 1989) and has been shown to protect against soman-induced cell death (J. Pharmacol., 198, 215, 1991). Preliminary studies with 15 mg/kg memantine also have shown to prevent seizures after soman (an irreversible cholinesterase inhibitor) injection (Toxicol. Appl. Pharmacol., 112, 95, 1992). The purpose of this study was to examine efficacy of memantine as a pretreatment drug in protecting rats from seizure-induced neuronal damage after soman. Adult male Sprague Dawley rats received pyridostigmine (0.13 mg/kg i.m.) and atropine methyl nitrate (5 mg/kg s.c.) 30 min before a single injection of soman (0.1 mg/kg s.c. equivalent to 0.9 LD₅₀ dose). Memantine (18 mg/kg s.c.) was administered 60 min prior to soman. At 24 hr rats were anesthetized with sodium pentobarbital (65 mg/kg i.p.) and transcardially perfused with buffered 10% formalin. Neuropathology of various areas of brain was examined in H&E stained coronal sections. Approximately 64% of rats receiving soman alone showed severe seizure activity and died within 24 hr. Pretreatment of rats with memantine reduced the severity of seizures and provided 100% protection from lethality. The brains of surviving rats in soman alone group showed necrotic lesions in frontal cortex, piriform cortex and hippocampus. Although memantine reduced the intensity of seizures in 66% of the pretreated rats, the drug could not protect the rats from seizure-induced neuronal degeneration in the brain areas described above. It is likely that repeated administration of memantine is needed to maintain the blockade of channels activated by excitatory amino acids in soman poisoning.


The recent molecular cloning of several NDMA subunits demonstrated the heterogeneity of NDMA receptor localizations and properties in the central nervous system. To determine oligonucleotides (AOs), we investigated the involvement of the NDMA₁ and the four NDMA₂ subunits in the formation of NDMA receptors which mediate excitotoxicity. Cerebellar granule cells displayed mRNA for these subunits after 10 days in culture as revealed by RT-PCR. Preliminary experiments showed that a maximum amount of stable OAs was incorporated inside cells at 24-48 h after transfection. Treatment of neuronal cultures with NDMAₙ and AOs decreased dramatically the level of NDMA₁ protein and reduced by 60% NDMA-induced excitotoxicity. We found that AOs, but not OAs for NDMA₂, 1-2 and 3 protected also neurons from NDMA excitotoxicity when 40 to 60% of cells were damaged by NDMA. However, only AOs for NDMA₁ and 3 displayed some protective effect when the NDMA-dependent neuronal death was reduced by 70%. AOs against NDMA₄ were much less efficient in rescuing neuronal cultures whatever the level of excitotoxicity. The protective effects obtained with AOs for NDMA receptor subunits were not additive. The reduction of neuronal cell death after AOs treatments was correlated with a decrease in the NDMA-stimulated calcium influx. Finally, NDMA AOs treatment did not affect the toxicity induced either by activation of AMPA glutamate receptor or by MPTP. These results suggest that AOs for NDMA subunits can be used to knock down the pathologic roles of specific NDMA receptor subpopulations in neuronal cultures.

TOXIC DOPAMINE/GLUTAMATE INTERACTIONS IN CULTURED NEURONS: EVIDENCE FOR OXIDATIVE STRESS. K.R. Hoyt*, L. Reynolds and T.G. Hastings. Dept. of Pharmacology and Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Dopamine (DA) and glutamate (Glu) are neurotransmitters important for normal brain function. However, these neurotransmitters can cause neuronal damage when their extracellular concentrations are elevated, in pathological conditions such as cerebral ischemia and methamphetamine toxicity, and possibly in neurodegenerative conditions such as Parkinson’s disease. We have found, using the fluorescent dyes CMFDA and monochlorobimane to measure intracellular glutathione, and dichlorofluorescein to measure intracellular oxygen, that evidence for DA and Glu-induced oxidative stress. We also investigated the interaction between DA and Glu on cell death in cortical neurons cultured for 14-17 days. We used concentrations and exposure times of DA and Glu that were themselves not toxic, and we found that the combination caused a significant increase in cell death. We found, using the fluorescent dyes CMFDA and monochlorobimane to measure intracellular glutathione, and dichlorofluorescein to measure intracellular oxygen, that evidence for DA and Glu-induced oxidative stress. We also investigated the interaction between DA and Glu on cell death in cortical neurons cultured for 14-17 days. We used concentrations and exposure times of DA and Glu that were themselves not toxic, and we found that the combination caused a significant increase in cell death.

These two treatments resulted in a 36% loss of viability (p<0.05). In another experiment, 100 µM DA for 1 hr or 100 µM Glutamate for 2 min resulted in a 4% loss of viability compared to untreated controls, when measured 6 h after treatment. When these two treatments were combined, there was a significant increase in cell death (p<0.05). The combined treatments were 0.5 µM DA for 2 h and 0.5 µM Glutamate for 2 min. These results suggest that the combination of DA and Glu can cause a significant increase in cell death. We have found, using the fluorescent dyes CMFDA and monochlorobimane to measure intracellular glutathione, and dichlorofluorescein to measure intracellular oxygen, that evidence for DA and Glu-induced oxidative stress. We also investigated the interaction between DA and Glu on cell death in cortical neurons cultured for 14-17 days. We used concentrations and exposure times of DA and Glu that were themselves not toxic, and we found that the combination caused a significant increase in cell death. We have found, using the fluorescent dyes CMFDA and monochlorobimane to measure intracellular glutathione, and dichlorofluorescein to measure intracellular oxygen, that evidence for DA and Glu-induced oxidative stress. We also investigated the interaction between DA and Glu on cell death in cortical neurons cultured for 14-17 days. We used concentrations and exposure times of DA and Glu that were themselves not toxic, and we found that the combination caused a significant increase in cell death.

This work was supported by MH18275 and MH41556.

It has been suggested that ischemia-induced CNS damage is mediated by prolonged activation of excitatory amino acid receptors which may result in whole or in part increased extracellular glutamate (tGLU). In these studies transverse hippocampal slices from male rats were used to investigate the effect of glycine (a-lo-taurodinitol) or hyposia (KCN) on temporal GLU release and associated neuronal damage mediated by glutamate (GLU) receptors. Treatment with a drug for 30 min increased extracellular aspartate (ASP) and GLU and produced lesions in the CA1, CA3, CA4 and dentate gyrus (DG) regions which were attenuated by NMDA antagonists. A more severe lesion was observed in slices treated for 30 min with both IOA and KCN; this was completely prevented by combining non-NMDA and NMDA antagonists. To investigate the temporal release of GLU and activation of GluRs, slices were treated with KCN for 2.5 to 30 min. Within 2.5 min discrete CA1 neuronal twiglave was seen which coincided with decreases in tissue ATP levels. These CA1 lesions were not accompanied by increases in extracellular GLU or ASP but were completely prevented by NMDA antagonists. Within 10 min there were increases in GLU which coincided with extensive lesions in the CA1, CA4 and DG. Within 20 min, all of the regions of the hippocampus appeared severely damaged. This was twice that of control slices. This data supports our previous studies which suggested that excitotoxicity associated with mild metabolic stress was caused by changes in the physiology of the NMDA receptor following compromise of energy stores rather than by increased extracellular GLU or ASP. More prolonged or more severe metabolic inhibition precipitates involvement of non-NMDA receptors perhaps mediated by increased tGLU.


Several a ligands have been shown to protect against neuronal ischemic injury in vivo. We have used primary cultures of rat spinal cord slices to evaluate the protective effects of a variety of selective a ligands in comparison with PCP-related noncompetitive NMDA receptor antagonists during either hypoxia or brief exposure to excitotoxic (100uM) concentrations of NMDA or kainic acid (KA). In addition, the effects of these compounds on NMDA-induced changes in intracellular calcium concentration ([Ca2+]i) and phosphorylation(s) (Pu) metabolism were compared. After 8-30 days in culture, neurons were subjected to hypoxia or brief excitatory amino acid exposure in Locke’s solution from which Mg2+ and glucose were omitted. Cell damage was quantitatively assessed on the following day by measurement of glutamate dehydrogenase (GluR). The same [Ca2+]i were measured in single identified neurons (5-20uM) [field] preloaded with the Ca2+-selective dye indo-1 using an ACAS 570A laser eyepiece. The following ligands MK-801, dextromethorphan, and a variety of e ligands, including DTG, (+)-pentamcine, and caramphine, caramphine, caused significant, dose-dependent protection against hyposia and NMDA, but not KA-induced cell injury. In contrast to the PCP receptor ligands, at neuroprotective concentrations the e ligands failed to antagonise the sustained elevations in [Ca2+] elicited by NMDA and failed to consistently alter NMDA-stimulated PI hydrolysis (200-500% above control). These results indicate that, through mechanisms distinguishable from PCP-receptor related antagonism of the NMDA receptor, a ligands provide an effective means of preventing excitotoxic neuronal injury.


NMDA antagonists such as MK801 and phencyclidine injure a discrete population of neurons in the cingulate and retrosplenial cortex. These agents produce cytoplasmic vacuoles and induce production of the stress protein HSP70. Cycloartenol arachidonic acid (AA) metabolites such as Pge2 and bosphenol A2 have been proposed to be extracellular messengers that induce HSP70 expression in tumor cells. Accordingly, we hypothesized that arachidonic acid metabolites may signal HSP70 expression due to MKO1 toxicity. MKO11mg/kg was injected i.p. in awake rats. HSP70 mRNA transcription was studied using in situ hybridization with 35S-labeled oligodeoxynucleotides (ODN). These ODN hybridized to HSP70 mRNA and protein expression in a dose dependent manner at 5, 10, 20, 30 and 50mg/kg i.p given 15 min prior to MKO1 injection. Furthermore, the mRNA encoding the mitogen inducible cycloxygenase (COX) was also induced. HSP70 in situ hybridization was also expressed 4 hrs after MKO1 injection. These data support a role for AA metabolites in the expression of HSP70 in MKO1 toxicity. Further experiments will be required to determine if AA metabolism inhibition in vivo or if their effects are limited to HSP70 induction.


Recent studies using the single-channel patch-clamp technique have shown that the 9-aminoacridines are potent open-channel blockers of NMDA-activated currents in cultured hippocampal neurons (Bullock, et al. J. Neurosci. 1989). In previous studies using the single-channel patch-clamp technique we have demonstrated that the 9-aminoacridines are potent open-channel blockers of NMDA-activated currents in cultured hippocampal neurons (Nelson, et al., Proc. Natl. Acad. Sci., USA 1994). Based on the acidine-induced reduction in single-channel open times at 800uM, the forward blocking rate constants were found to be 1.1±0.7 10^3 s^-1 and 1.4±0.3 10^4 s^-1 for 1,2-phenyl-4-9-aminoacridine (1,2-PAA) and 3-[2-9-aminoacridinyl]propionic acid (IOA-iodoacetate) (IOA), respectively. The affinity of the 9-aminoacridines for the blocked state produced by these compounds was long in duration (in the range of 200 min) similar to that of normal channel closed times. To circumvent this problem, experiments using whole-cell patch-clamp preparations were performed. A two-metal, multielectrode configuration was used to study the binding kinetics of these compounds. In nominally Mg2+ free solutions, the recovery from blockade by THA indicated that the dissociation was from a single blocked state (k_diss=48.7±0.9 min^-1, n=3), but the single-channel experiments clearly showed that a much briefer THA-blocked state (dissociation <200 µs) exists that would be difficult to detect by any means of drug application in the whole-cell experiments. The recoveries from blockade by the bis-9-aminoacridines, on the other hand, were slower and were normally best described by a single exponential function having time constants (k_diss) of 2176.1±14.7 min^-1 (n=5), and 1671.8±9.9 min^-1 (n=12) for 1,2-PAA and 1,4-BAA, respectively. The single-channel studies mentioned above also revealed that the interaction of the 9-aminoacridines with the channel was at a site that is different from the Mg2+-binding site of the channel and suggested that the channel could be similarly occupied by 1,4-BAA and the 9-aminoacridines. This simultaneous occupation likely modifies the affinity of the receptor for the 9-aminoacridines. Indeed, preliminary results in the presence of 100µM 9-AAC showed that the ratio of association and dissociation of 1.2-PAA were increased. Support (USPHS Grant ES 07330).

628.18 NEURO NECROTIZING PROPERTIES OF PHENCYCLIDINE TO CORONE. DE Worum, MA Sanna, and JW Olwayo. Washington University, St. Louis MO 63110.

Antagonists of NMDA glutamate receptors, including phencyclidine (PCP) and MK-801, acutely injure neurons and induce expression of 72 Kd heat shock protein (HSP) in the posterior cingulate/retrosplenial (PCRS) cortices when administered sc to adult rats. Sharp et al. reported that a high dose of PCP (40 mg/kg) caused a robust HSP response in neurons but in many additional neurons in several neocortical and limbic brain regions. They proposed that HSP serves a protective function and that the robustness with which an injured neuron expresses HSP may be a measure of its ability to survive the injury. Thus, the widespread injury induced by high-dose PCP may be entirely reversible. In contrast, it is known that injury to MK-801 (5 mg/kg ip) kills PCR S neurons. Silver stains have been used to detect neuron injury induced by low-dose MK-801, but h to date it is not known that silver staining of neurons signifies cell injury or cell death. To address these questions we treated adult female rats with a single high dose of PCP (50 mg/kg) and examined the brains at various post-injection times (4 hrs to 4 days) using H&E paraffin, de Olomos silver or HSP immunocytochemistry. We found high dose PCP induced HSP in PCR S neurons in layers II to V and that it killed many neurons distributed in these same layers. Occasionally, but not consistently, it induced HSP and killed neurons in other neocortical areas. We have been unable to validate the de Olomos silver stain for detecting PCP-induced neuronal necrosis in that in each brain only those neuronal populations showing definite signs of neurons (intense eosinophilia) stained with de Olomos stain. Moreover, the de Olomos stain may be a selective marker of cell death in that in PCR S neurons did not become argyrophilic until approximately 36 hrs after PCP treatment, but PCP is well known to physically injure neurons at a much earlier interval (4-12 hrs). Our findings do not confirm the view that HSP expression in PCP-injured neurons signifies necrosis. Supported by AA 07666, AG 05681, DA 05072 and RSA MH 38894 (FWO).
RATS BECOME HYPOSENSITIVE TO MK-801 NEUROTOXICITY DURING PREGNANCY. NB Farber* and JW Chelley Washington Univ., St. Louis, MO.

Antagonists of NMDA glutamate receptors, including phencyclidine (PCP) and MK-801, cause vascular injury (low dose) or kill (high dose) neurons in the posterior cingulate/retrosplenial (PCR/S) cortices when administered to or ip to adult rats. Non-pregnant female rats are substantially more sensitive to the vasculotrophic effects of these antagonists than non-pregnant males. We now report that female rats become hyposensitive to NMDA antagonist neurotoxicity (NAN) during pregnancy. A high dose of MK-801 (5 mg/kg sc) in 17/19 pregnant dams (n = 4) induced a vascular reaction affecting 88.75 ± 25.8 (SEM) neurons per section compared to 212.2 ± 18.9 (SEM) neurons per section for control pregnant females (n = 4). In the female pregnant dams, some microglial proliferation and astrocytosis were observed. In contrast, we administered MK-801 to adult female rats in a dose (0.5 mg/kg sc) that reliably induces a full cerebrocortical neurotoxic reaction and injected various agents into the PCRs cortex and other brain regions in attempts to disrupt the circuit and block the PCRs reaction. We found that the neurotoxic reaction could be prevented by pretreatment with remifentanil (GABA antagonist), scopolamine (muscarinic antagonist) or nimodipine (Ca2+ antagonist) into the PCRs cortex, or by injecting nimodipine into the diagonal band (DB) region of the basal forebrain, or into the anterodorsal/ventromedial (ADAV) nucleus of the thalamus. Our interpretation is as follows: systemic administration of MK-801 blocks NMDA receptors that tonically drive GABA inhibitory neurons in at least three separate brain regions (PCRs, DB, ADAV). This results in a complex disorganization syndrome in which 1) DB neurons release excessive ACh at muscarinic receptors on PCRs neurons. 2) ADAV neurons release glutamate at non-NMDA receptors on PCRs neurons. 3) local PCRs interneurons release an endogenous agonist that modulates a sigma site in PCRs cortex. We postulate that the proximal mechanism causing PCRs neuronal injury is simultaneous excessive release of 3 endogenous transmitter/modulator systems that can induce these anomalies. Supported by NIH NS 8254 (LWO) and a grant from NARSAD (JWO).

EXCITATORY AMINO ACIDS: EXCITOTOXICITY AND NON-NMDA RECEPTORS

629.1 KAINATE FAILS TO EVOKIE MITOCHONDRIAL OXIDATIVE RADICAL GENERATION IN KAINATE-LACERATED CORTICAL NEURONS: SELECTIVELY VULNERABLE TO AMPA/KAINATE TOXICITY, L.M.T. Canzonier*, S.L. Sensi, L.D. Dugan, D.M. Turkasy, and D.R. Chi. Dept. of Neurology, and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

We previously described a subpopulation of cortical neurons highly vulnerable to death induced by relatively brief AMPA or kainate exposure, identifiable by staining for kainate-stimulated calcium uptake ("kaout+ positive cells"; Turkasy et al., Soc.Neurosci. Abstr. 18.81, 1992), a marker for cells expressing AMPA or kainate receptors. Herein, we report data from a separate, earlier study. We assessed whether "kaout+" cells, followed by death of >50% of the cells. The increase in [Ca2+]i was comparable to that reached following neurotoxic exposure to NMDA (100 mM for 5 min) in the general neuronal population. However the same kainate exposure failed to stimulate mitochondrial oxidative radical production in cobalt-positive cells (n=19). Failure to detect a rhodamine signal was not due to loss of the mitochondrial membrane potential as tetramethylrhodamine showed no loss of this potential in cobalt-positive cells. Present data suggest that the route of Ca2+ entry may be an important determinant of consequent mitochondrial oxidative radical production. Supported by NIH grants NS33073 (DWC) and AG05099-01A1 (L.DD).

629.2 IMAGING OF MITOCHONDRIAL OXIDATIVE RADICAL PRODUCTION IN CORTICAL NEURONS EXPOSED TO AMPA AND KAINATE. L.D. Dugan, S.L. Sensi, M.T. Canzonier, M.P. Goldberg, S.D. Handran, S.M. Rothman and D.R. Chi. Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110.

Confocal microscopy was used to image oxidative radical generation by mitochondria in cortical neurons exposed to NMDA. The mitochondria-specific, oxygen-sensitive dye, dihydroethidium (DHE; 100 nM) was added to cultured cortical cells for 30 min. A time- and dose-dependent increase in mitochondrial fluorescence was observed in response to toxic concentrations (50-100 μM) of NMDA. This signal was only observed after onset of NMDA exposure, reflects oxygen radical-mediated oxidation of non-fluorescent DHE to its fluorescent derivative, rhodamine (2D).

The signal was specific to NMDA in that 100 μM kainate (10 μM MK-801), 50 μM potassium (10 μM MK-801), 1 μM ibotenic, or 300 μM t-ACPD all failed to stimulate mitochondrial radical formation. Removal of calcium (plus 300 μM EGTA) abolished the NMDA-stimulated mitochondrial response. In an uncooled of electrophoresins from a non-NMDA group that did not sensitively increase mitochondrial radical production. Supported by NIH NS33073 (DWC), AG05099-1A1 (L.D.), NS51345 (MPD).


SyM-32 is a monocular antibody (Stemberger-Meyer Immunocytochemistry) that recognizes non-phosphorylated neurofilament epitopes, and has been reported to label subsets of CNS neurons, including populations of pyramidal neurons that degenerate in Alzheimer’s disease (J Comp Neurol 301:44). We found SyM-32 immunostaining to label small subsets (2-4%) of neurons in dissociated cultures of murine cortex and spinal cord. Cortical SyM-32 (+) neurons were larger than average, frequently multipolar. Immunocytochemistry using a polyclonal antibody to GluR4 revealed moderate levels of receptor expression in 80-90% of cells. Fura-2 microfluorometry demonstrated that either AMPA (100 μM) or kainate (100 μM) increased [Ca2+]i, approximately 1 fold over basal, in the transfected line. Addition of 100 μM cyclothiazone, which blocks AMPA receptor desensitization, to the exposure medium increased the AMPA-induced elevation in [Ca2+]i, to about 10 fold; further increases could be blocked by raising the extracellular Ca2+ concentration from 1 mM to 10 mM. Continuous exposure to 500 μM AMPA, 100 μM cyclothiazone, and 10 mM Ca2+ resulted in the death of 50-60% of transfected cells in 1 hour, and about 90% of cells by 48 hrs. Death was dependent on AMPA concentration (30-1000 μM), and could be blocked by 50 μM NBQX. Death could also be induced by 24 hr exposure to 500 μM kainate, but not by 48 hr exposure to 500 μM NBQX. Supported by NIH grant NS33073 (DWC).
AMP-INDUCED DELAYED NEURONAL INJURY IN THE HIPPOCAMPAL SLICE, WITH PROTECTION WITH POST-TREATMENT DNXO. K.L. Peterson and D.A. Watts. Dept. of Neurology UCLA, Los Angeles, CA 90024 and Sepulveda VAMC, Sepulveda, CA 91343.

Prevention of delayed neuronal injury is a prime objective in the acute treatment of stroke and head trauma. To investigate potential non-NMDA excitotoxic mechanisms of delayed neuronal injury, we examined the effect of sub-lethal AMPA exposure upon CA1 neurons in hippocampal slices. In these studies, paired rat hippocampal slices taken from the same dissection were placed in recording chambers perfused with ACSF containing 2.4 mM Ca++, 1.3 mM Mg++ and 4 µM glutamate. One slice of each pair was exposed to 25 µM AMPA until disappearance of the orthodromic CA1 population spike (PS). In three experiments, the AMPA-exposed PS was 3.7 ± 0.9 ms in duration. AMPA treated slices regained an average of 98% ± 2 of their original orthodromic PS within a mean of 30 ± 10 min. After initial recovery, AMPA-treatment resulted in significantly smaller PS amplitudes for several hours and then abruptly collapsed. Control slices maintained PS significantly longer. A 3nM CA1 PS amplitude was used as the criterion for slice viability. AMPA treated slices maintained a PS of greater than 3 mV for an average of 6.3 ± 1.4 hrs while paired control slices maintained a PS greater than 3 mV for 16.5 ± 1.1 hrs. Post-AMPA treatment of 100 µM DNXO extended the duration of CA1 PS recordings from 7.7 ± 0.4 hrs to 18.6 ± 1.4 hrs. These findings suggest that activation of non-NMDA receptors can induce delayed neuronal dysfunction, and that protection from this injury can be afforded by DNXO treatment given after the receptor activation.

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Domino acid (DOM) is a rigid analog of glutamate demonstrated to be an agonist on non-NMDA receptors. We previously demonstrated that DOM given i.p. to mice induces the c-fos immediate response gene and causes degeneration in hippocampal CA1 and CA2 pyramidal cells. We have now investigated the acute effects of DOM on neuronal viability, using rats aged postnatal (P) day 1, 5, and 10. DOM-induced behavioral changes include immobility and ataxia, repetitive scratching and finally generalized clonic or tonic-clonic seizures. P1 rats were more sensitive than P10 (EC50 = 0.3 to 0.7 mg/kg). Scratching occurred within 20 min and seizures within 30 min at 0.2 mg/kg, twice as long at 0.1 mg/kg and did not occur at 0.02 mg/kg. We next localized the in vivo action of DOM to hippocampal pyramidal cells of CA2 and CA3 using c-Fos immunohistochemistry. Primary hippocampal cultures were then used to evaluate the toxic effects of DOM. 30 µM DOM exhibited [Ca2+]o, and caused membrane depolarization measured using FURA-2 and bisoxinol. The NDMA antagonist AP-5 (100 µM) totally prevented DOM elevated [Ca2+]o and depolarization. Pretreatment of PS rats with 1 mg/kg AP-5 also prevented the induction of c-Fos by DOM, but failed to prevent DOM-induced seizures. These results indicate that neonatal rats are highly sensitive to the effects of DOM. The tonic and neuroexcitatory actions of DOM on pyramidal cells appear to require involvement of NDMA receptors, yet the behavioral changes appear to occur independently of the NDMA receptor subtype.


We and others have shown that in primary neuronal cultures, neuroprotective agents which do not block glutamate receptors protect from excitotoxicity by selectively affecting mechanisms associated with either non-NMDA or AMPA receptors or non-NMDA receptors (Brain Res 1993, 292; 304). Thus, non-NMDA, but not the NMDA, excitotoxicity can be attenuated with an analog of vitamin E, suggesting that free radical formation plays a role in the latter type of toxicity (Brain Res 1991, 588). Recently, it was proposed that melatonin might be a potent endogenous hydroxy radical scavenger (Endocrine J 1993, 11). In addition, melatonin has been shown to protect neurons from photochemically induced oxidative stress (Mol. Brain Res. 1985, 2: 193). In this study, we used 7-day-old primary cultures of rat cerebellar granule neurons. Cultures were exposed to kainate (30 µM), glutamate (15 µM), or NMDA (without magnesium, 80 µM) and then returned to the culture-conditioned medium. Viability was measured with the MTT technique (Neuropsychopharmacology 1990, 29: 1103) 18-16 hrs later. Co-treatment with melatonin (500 µM) protected neurons completely from the toxicity of kainate (up to 1 µM), and shifted the LD50 for glutamate from 5 µM to 97 ± 6 µM. It was ineffective in protecting from NMDA toxicity. When melatonin was added to cultures only before or after kainate treatment, there was no resultant protection from kainate toxicity. The neuroprotective effect of melatonin does not appear to be related to the direct action of melatonin on non-NMDA glutamate receptors. That is, the kainate-stimulated increase in the cytosolic calcium (measured at the single cell-level with a digital imaging fluorogenic microscopy with fura-2) was not affected by melatonin; the binding of SH-glutamate to rat cerebellar membranes was likewise not affected. Further studies are needed to evaluate the pharmacological relevance of the neuroprotective action of melatonin.
629.11

Kainate, a non-NMDA receptor agonist, acts as a potent neurotoxin in the adult retina. Consistent with this, acute exposure (12 hours) of primary cultures of chick embryonic day 8 retinal cells to 100 μM kainate caused 59 ± 5% of the cells to die when applied at 6 days in vitro (DIV). In contrast the number of cells in cultures grown in the presence of 1.0 and 500 μM kainate from 1 DIV did not differ significantly from those grown in the absence of kainate. This lack of effect of kainate on cell survival does not appear to result from receptor desensitization since whole-cell patch-clamp recording showed that cells grown in the presence of kainate for 6 days continue to exhibit a current which is suppressed by the non-NMDA receptor antagonist CNQX. We have utilized the cobalt staining technique of Prus et al. (1991, Neuron 7, 309) to determine the number of cells that express the calcium permeable form of the AMPA/kainate receptor in retinal cultures. We found that the number of cells expressing this receptor in control cultures rose from 5% at 2 DIV to a maximum of 50% at 3 DIV. However, in cultures grown in the presence of 10 μM kainate, the number of cells stained by cobalt was reduced by as much as 90% at 5 DIV. The effects of kainate on the expression of the receptor could be prevented by the addition of 20 μM CNQX, but not by 20 μM AP5 or 20 μM D/7 or dinitrophenol. When kainate was removed from the culture medium at 4 DIV the number of cells expressing the receptor at 7 DIV was restored to normal. A down-regulation of the Ca2+-permeable non-NMDA receptor will decrease Ca2+ influx into the cells and this may account for their survival when grown in the presence of excitotoxic concentrations of kainate.

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629.13
INTRINSIC OPTICAL SIGNALLING IN THE HIPPOCAMPAL SLICE EVOKED BY THE EXCITOTOXIC DOMINO ACID. Trevor M. Poliouchou* & David Andrew. Dept. of Anatomy & Cell Biology, Queen’s Univ., Kingston, Ontario, K7L 3N6.

As cells swell, light transmittance increases across brain slices (Lipton, J. Physiol. 237, 365; Andrew and MacVicar, Neuroscience in press). Also, as shown by Adams and Andrew (this meeting), increases in light transmittance induce by glutamate agonists can be imaged in real time in the entire hippocampal slice. Here we studied the effects of the potent neurotoxin and glutamate analogue, domino acid (D/7) on light transmittance in the CA1 hippocampal slice. The aim was to characterize 1) which hippocampal regions were affected, 2) which subclass of receptors were involved, and 3) if cell swelling played a role in the response. A 1 minute exposure of 10 μM D/7 (25°C) elevated light transmittance by up to 58% in the dentate regions of CA1 and of upper dentate gyrus. The response slowly reversed during a 30 min wash in –NMDA. A significant change was observed in the CA1 region nor in the lower blade of the dentate gyrus. The response to D/7 was reversibly blocked by the non-NMDA receptor antagonists kynurenic acid (1 mM; n=5), CNQX (50 μM; n=5) or D/7 alone (1 mM; n=5) or the kainate receptor antagonists GAMS (100 μM; n=5). Tetrodotoxin (1 μM) blocked action potentials and the evoked CA1 population spike but light transittance increases were maintained and therefore were not associated with neuronal discharge. Extracellular tissue resistance measured across CA1 stratum radiatum increased rapidly in response to DOM and slowly fell over 30 min, which paralleled the light transittance response. We conclude that brief DOM exposure elicits a prolonged, post-synaptic swelling in the CA1 dentitid region primarily mediated by AMPA receptors. This imaging technique allows a real-time view of events preceding neuronal death and so may prove useful in assessing potentially therapeutic glutamate antagonists that combat excitotoxicity. Supported by the Canadian MRC.

629.14

Experimental primate studies have shown that chronic injection of the excitatory and neurotoxic amino acid BOAA, disrupts motor neuron function in a manner similar to that seen in the human neurodegenerative disease, amyotonia. BOAA is known to exert its neurotoxic effects by binding to both NMDA and non-NMDA receptors, especially the AMPA receptor. A recent study (Brain Res., 621:215, 1993) has reported that a very low (0.1 μM) concentration of BOAA selectively inhibits the activity of the mitochondrial enzyme NADH-dehydrogenase (NADH-DH; Complex 1), while 1 μM BOAA promotes LDH release into the incubation media indicating neuronal cell death. This study investigates the effect of BOAA on mouse brain NADH-DH in both vitro and in vivo. For in vitro studies, transverse brain slices were obtained from adult male CD-1 mice (25-34 g) and incubated in Krebs Ringer (3.0 ml/slices) bubbled with 95% O2 + 5% CO2 incubated at 37°C for 1 h. Treatment of slices with BOAA showed a concentration-dependent inhibition of NADH-DH, but only at μM concentrations (16, 24, 33, and 37% inhibition at 10, 100, 300 and 1000 μM BOAA, respectively). Release of LDH into the incubation media was observed only in slices treated with 500 μM and 1000 μM BOAA. Young (9-day old) and adult (4-month) mice treated with 500 and 700 mg/kg BOAA, respectively, showed behavioral signs of BOAA toxicity. Young mice had severe convulsive seizures while adult mice exhibited drowsiness and lethargic movement. The NADH-DH activity in brain homogenates and mitochondria from BOAA-treated animals did not differ with that in age-matched animals treated with an equivalent volume of saline. These findings are in contrast to the reported suggestion that BOAA is a highly potent inhibitor of mitochondrial energy metabolism. Supported by NIH grant NS 19611 and MRG of Oregon.

AMPA receptors are characterized by a fast desensitization which is considered to be a possible mechanism for the termination of excitatory synaptic transients. The present experiments are aimed to evaluate a methodology of cyclothiazide (CTZ), a drug which reduces desensitization of AMPA responses, to alter the intraneuronal Ca++ concentration ([Ca++]i) after exposure to AMPA.

Experiments were performed in primary rat hippocampal neurons grown for 10-16 days in cultures. Changes in [Ca++]i were measured by microfluorimetric monitoring of the fluorescence intensities from individual neurons loaded with fura-2. In these cultures, indirect immunostaining of rat MAP2 confirmed that the fluorescent signal was derived from neuronal cells of the neurite, which excited most of the neuronal responses. The effects of Concanavalin A (Con A) on the pharmacology of glutamate were examined. In cultured cerebellar granule cells, we found that Con A increases Ca++ influx through the MAP2 buffer, glutamate, quinolinic acid, and a-methyl-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) activate the same neural subset population (10-15%) of the neurons, whereas quinolinic acid activates all neurons and NMDA activates none. Glutamate, AMPA and quinolinic acid decrease the kainate-induced elevations of [Ca++]i in cells unresponsive to glutamate (80-90%). The treatment of cells with Con A (1) reverses the quiescent inhibition of the kainate response, (2) recovers the percentage of neurons responding to glutamate, AMPA and quinolinic acid without increasing calcium and decreasing NMDA responses, (3) and results in the pharmacological expression of a specific population of receptors. Finally, Con A-like effects are seen in co-cultures of neurons and glia. Contrary to the assumption that glutaemate is universally excitatory to neurons, these results indicate that the neuronal response to glutamate is not apparent in most neurons in cerebellar neuronal cultures and that glia, like Con A, can change the glutamate pharmacology.

630.2 INTERACTIONS OF GYKI 52466 AND CYCLOTHIAZIDE: PATCH CLAMP STUDIES. G. Ramps, W. Miller*, D. Swadulla, C. G. Parsons* and G. Collingridge*.

The effects of GYKI 52466 and cyclothiazide on isolated AMPA receptor-mediated EPSCs and AMPA-induced currents were investigated using current and patchclamp recordings from the CA1 region of hippocampal slices and cultured rat hippocampal neurons. Cyclothiazide at the concentrations sampled (10-30pM) reduces saturation uptake of Ca++ in rat hippocampal slices and cultured rat hippocampal neurons. The effects of cyclothiazide on calcium currents were investigated using a Ca++ imaging protocol to identify the effects on calcium currents activated by AMPA, quisqualate, and kainate. Cyclothiazide (10-30pM) reduces the amplitude of AMPA-EPSCs with little effect on AMPA-IPSCs. The effects of cyclothiazide on calcium currents were investigated using a Ca++ imaging protocol to identify the effects on calcium currents activated by AMPA, quisqualate, and kainate. In conclusion, cyclothiazide may be a antagonist and GYKI 52466 as an agonist at a common regulatory site on AMPA receptors but the two compounds modulate different effects on receptor activation kinetics. Furthermore, these data provide supportive evidence that the offset kinetics of AMPA receptor-mediated EPSCs are partially governed by desensitization.
2,3-BUTANEDIONE MONOXIME SUPPRESSES KAINATE-INDUCED CURRENTS OF MRUNE VENTROMEDIAL HYPOTHALAMIC NEURONES. J.H. Ye* and J.J. McAdoo, Dept. Pharmacology/Toxicology, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, N.J. 07103-2714.

The effects of the "chemical phosphate", 2,3-butanedione monoxime (BDM) on kainate-induced current were studied in hypothalamic neurones acutely dissociated from young mice. Drug containing solutions were rapidly applied to activate the neuron and were subsequently subjected to the system perfused with patch or conventional whole cell recording techniques. When applied simultaneously with kainate, BDM produced a rapid, reversible and dose-dependent (Na+) block of kainate-induced current. This acute blocking effect of BDM was neither voltage- nor use-dependent. Including 500 μM ATP-gamma-S in the bath reduced the apparent effect of BDM. Furthermore, H-7 (200μM), a non specific protein kinase inhibitor, did not prevent the rapid recovery of the kainate response following washout of BDM. These findings suggest that the acute blocking action of BDM on the kainate response of hypothalamic neurones requires a "chemical phosphate" action. Support from the NIH (NS31040) and the nIHAAN (AA80825) made this work possible.

630.9 KAINATE-INDUCED Ca²⁺ SIGNALING IN ASTROCYTES REQUIRES Na⁺ AND Ca²⁺ FLUX VIA THE Na⁺/Ca²⁺ EXCHANGER. W.T. Kim, M.G. Reulat, and A.H. Comell-Bell*. Dept. of Cell Biology, Yale Univ. Sch. of Med., New Haven, CT 06510.

In primary cultures of rat hippocampal astrocytes, the binding of ionotropic glutamate receptors with kainate, kainate (KA, 100 μM) induces a rise and sustained elevation of [Ca⁺]ᵢ as well as regenerative intercellular Ca²⁺ waves. Using time-lapse confocal scanning laser microscopy with Fluo-1, we found that the removal of Na⁺ or Ca²⁺ from the bath abolished these [Ca⁺]ᵢ changes; therefore, external Na⁺ and Ca²⁺ are both necessary for the KA-induced response (N = 7 experiments). However, the [Ca⁺]ᵢ elevation in the ionotropic response is not via influx through voltage-dependent channels. Blocking Na⁺ channels with TTX (10 μM) did not affect the incidence, latency, or spatio-temporal characteristics of KA-induced [Ca⁺]ᵢ changes (N = 3 experiments). Similarly, blocking L-type Ca²⁺ channels with Nifedipine (10 μM) and Nimodipine (1 μM) did not affect the [Ca⁺]ᵢ elevation or waves (N = 3 experiments). However, inhibiting the Na⁺/Ca²⁺ exchanger with Benzamil (100 μM) completely abolished the [Ca⁺]ᵢ elevation and intercellular Ca²⁺ waves (N = 7 experiments). The inhibition of Ca²⁺ signaling by Benzamil appears to result from its effects on the Na⁺/Ca²⁺ exchanger in particular rather than to its non-specific effects on Na⁺ and Ca²⁺ channels and the Na⁺/H⁺-antipporter. The more selective Na⁺/H⁺-antipporter inhibitor 5-N (dimethyl)-amiloride (100 μM) did not affect KA-induced [Ca⁺]ᵢ changes (N = 3 experiments).

630.11 MK-801-INDUCED HYPERMETABOLISM IN THE POSTERIOR CINGULATE CORTEX IS ATTENUATED BY AN AMPA RECEPTOR ANTAGONIST. T.R. Petel, J. C. McCluskey*, Mellone Surgical Institute, University of Georgia, G611, Q.U. MK-801-induced increases in glucose utilization in the posterior cingulate cortex have been examined. A pretreatment with the AMPA receptor antagonist NBQX alters the pattern of glucose use seen with MK-801. Local cerebelar glucose use was measured in male 50 rat using stereotaxic-laser-microglucosography (70 mg/kg i.v.) was administered 2 min prior to the administration of MK-801. The combined effect of MK-801 on glucose use was assessed by examining 60 rats 24 h after treatment with NBQX. MK-801 induced a decrease in glucose use in the posterior cingulate cortex (30% decrease/10 min). Pre-treatment with NBQX attenuated the MK-801-induced decrease in glucose use in the posterior cingulate cortex (15% decrease/10 min). This suggests that MK-801-induced decreases in glucose use are mediated by AMPA receptors.


[³H]Kainate binding to rat cortical membranes can be separated into high- and low-affinity binding sites with Kᵣ values of 2 and 24 nm, respectively. The low-affinity binding site can be studied separately in the presence of Ca⁺⁺. Saturation of [³H]Kainate binding to rat brain sections in 10 mm CaCl₂ containing medium results in the presence of 30 nm CaCl₂. These results suggest that the use of CaCl₂ in the presence of both high- and low-affinity [³H]Kainate binding sites can be determined in all the investigated regions, whereas only high-affinity binding receptor population (Kᵣ = 400 μM) could be detected in 30 mm CaCl₂. Comparison of the regional distribution of the low-affinity [³H]Kainate binding site and the [³H]AMPA binding sites did not indicate that calcium insensitive [³H]Kainate binding represents binding to the AMPA receptor. The non-specifically bound [³H]Kainate in the presence of 30 mm CaCl₂ is inhibited by co-administration of NBQX, a competitive antagonist of the low-affinity binding site. The high-affinity [³H]Kainate binding site is insensitive to the presence of NBQX. The high-affinity binding site is insensitive to the presence of NBQX, but is sensitive to the presence of low-affinity binding site. The combination of these two sites may provide a useful tool for characterizing the potential adrenergic effect of the non-competitive NMDA receptor antagonists.
630.13

STRUCTURAL ANALOGUES OF THE NON-NMDA RECEPTOR ANTAGONIST
GYKI 52466 DEPRESS K+ CURRENTS IN HIPPOCAMPAL NEURONS.
W. L. D. L. Celli and L. K. Simmons. CNS Research, Lilly Research
Laboratories, Indianapolis, IN 46285.

The 2,3-benzodiazepine GYKI compounds have been characterized as
potent, selective antagonists of neuronal responses mediated by
non-NMDA receptors. In this study we used whole-cell voltage-clamp
protocols to investigate the K+ channel actions of structural analogues
of the parent compound GYKI 52466 in embryonic rat hippocampal
neurons.

Three of the four compounds evaluated blocked voltage-gated K+
currents with a rank order potency and stereoselectivity comparable
with their reported antagonist activities at non-NMDA receptors. In
contrast, the most potent K+ channel blocker of the compounds tested was
GYKI 53391, a 4′,3′-d-N-acetyl derivative of GYKI 52466
known to be inactive at non-NMDA receptors. Ymuranecate, CNQX and
Ro-15-1788 failed to elicit similar responses, therefore the GYKI
analogs do not appear to be modulating K+ channel function indirectly
via interactions with ionotropic EAAs or benzodiazepine receptors.

The results described here suggest that the K+ channel and non-
NMDA receptor sites of the 2,3-benzodiazepine GYKI compounds can be
isolated so that further structural analysis within this series may lead to the
identification of more potent K+ channel blockers.

630.14

DIFFERENTIAL RESPONSES TO PHOSPHONATE ANALOGS OF GLUTAMATE DISTINGUISH TWO SUBTYPES OF L-AP4-
Dept. of Biochemistry, Univ. of Minnesota, Minneapolis, MN 55455.

In previous studies of glutamate receptors, it has been shown
that L-2-amino-4-phosphonobutanoic acid (L-AP4) is a potent
inhibitor of excitatory glutamatergic projection pathways of the lateral
perforant pathway (LPP) as well as the CA3-CA1 synaptic pathway
(MF) of the guinea pig, and a partial inhibitor (10-40%) of the medial
perforant pathway (MPP) of the rat hippocampus. It is also a potent
inhibitor of the Schaffer collateral-CAL pyramidal pathway after
(but not before) sensitization by exposure to quisqualic acid (QUIS). It
was also demonstrated that the QUIS-sensitized site is pharmacologically
different from the L-AP4 receptor of the rat LPP and the guinea
pig MF. Specifically, the QUIS-sensitized site also shows high
sensitivity to L-AP4 and E-cyclopentyl-AP4. We now wish to report that
the partial sensitivity of the rat MPP also shows the pharmacological
profile of the QUIS-sensitized site, not of the classical L-AP4 receptors
which have low sensitivity for L-AP4 and E-cyclopentyl-AP4.

Exposure of a slice to QUIS sensitizes the MPP for 100% inhibition by
L-AP4, L-AP5, and E-cyclopentyl-AP4 with IC50 values indistinguishable
from the partial inhibition observed with these compounds before
QUIS. The high sensitivity of the rat LPP and guinea pig MF to L-AP4 proves
impossible to make it impossible to demonstrate QUIS-sensitization of
these pathways. However, using L-AP4 and E-
cyclopentyl-AP4, QUIS-sensitization can be readily demonstrated.

(Supported by NIH NS 17944).

631.1

PEPTIDES: BIOSYNTHESIS, METABOLISM, AND BIOCHEMICAL
CHARACTERIZATION II

631.2

CONVERSION OF MET-ENKEPHALIN-ARG-PHE TO MET-ENKEPHALIN
IN HUMAN SERUM. M. K. Lacy, D. J. O'Connor, N. Dow, and
Neuroscience Research Institute and Department of Chemistry, SUNY/Old
Westbury, Old Westbury, NY 11582-0210.

Both met-enkephalin (MKE) and the opioid peptide, Met-
enkephalin-Arg-Phe (YGGFRMF), are reported to be capable of activating
the same population of immune cells in the human circulatory system.
Earlier studies also showed that MKEGFPRMF is capable of binding to
a product with the same HPLC retention time (RT) as 125I-YGGFRMF. The
aim of this study was to verify the conversion of YGGFRMF to YGGFRMF in
human serum. Time-course studies were performed in the presence
(Petri-dish, WI) showed YGGFRMF (50 uM) was processed rapidly to three
major products with HPLC RT’s corresponding to that of 125I-YGGFRMF
and YGGFRMF. The material with the RT of YGGFRMF rose to its highest
level in 1 min and remained at over 70% of this level after 5 min. The
identity of the peptide material in this peak was verified with three
procedures. 1) It was reactive against YGGFRMF antibody in RIA. 2) When
derivatized with PTH the peptide derivative migrated with the same RT as
the derivatized YGGFRMF standard. 3) Amo acid analysis of the peptide with
PTC technique showed the presence of Y, G, F, R and M in the ratio of 1:2:1:1
as with YGGFRMF. Serum protease inhibitors studies showed the conversion
of YGGFRMF to YGGFRMF is mediated by ACE and GGFPRMF and GGFPRMF
are products of the action of aminopeptidase on YGGFRMF and YGGFRMF.

Unlike YGGFRMF, the opioid octapeptide, Met-enkephalin-Arg-Gly-Leu,
is not converted to YGGFRMF in human serum. Our results showed YGGFRMF
is converted to YGGFRMF in human serum and it may be serving as a part
of a mechanism for sustaining YGGFRMF concentration in the circulatory
fluid. Supported by NDA DA-0010, MNI-NIDA-COR NN-17138, and NIH
GM-08180.

631.3

COMPARATIVE POTENCIES OF KID-FORBIDDEN CALCIUMION
GENE-RELATED PEPTIDE ANALOGUES IN MUTYPOTOXICOSIS.-
GFP, AND COQ3 IN VITRO BIODISY. S. L. Letyash, J. D. Bond, D.
Joakim, L. S. Escobedo, A. Foumier, and R. D. Diamond (1) Douglas
H3H 1R3. (2) INS-Sante, Place-Clair, P.O. Box, Canada. H3R 1G9.

Alienes substituted analogues and Fragments of NCPPR, NCPPR, NCPPR, (a potent
antagonist) and its linear analogue [cys(ACh)]2-CPPGR (a CGP, preferential
antagonist) were synthesized and the respective role of Glu acid residue between positions 14 to 23 for their abilities to induce an
toxic effect in the guinea pig al初心 and inhibition or contraction of the
electrically stimulated rat vas deferens, two prototypic CGP, and CGP,
receptor biosays, respectively (Demini et al., J. Pharmacol. Exp.
Ther. 1982: 206: 123-129. 1982). Results demonstrated that L-Leu, Leu and Arg are critical for the activation of CGP receptors in both tissues: [cys(ACh)]2-CPPGR being inactive. Instead, substitution of Glu residue in NCPPR, NCPPR, NCPPR, (a potent
antagonist) for Cys residue in [cys(ACh)]2-CPPGR and [cys(ACh)]2-CPPGR
might be a part of the CGP preparation while being somewhat less potent (69%) than the endogenous counterpart. The two analogues [cys(ACh)]2-CPPGR and [cys(ACh)]2-CPPGR, were slightly more potent than the unmodified analogue on CGP receptors whereas the CGP, receptor activity of the CGP, receptor activity of Glu substitutions for Cys (ACh)2-CPPGR, [cys(ACh)]2-CPPGR, and [cys(ACh)]2-CPPGR, displayed higher
potencies than L-Leu, Leu, and Arg in the CGP, antagonist site, while being ineffective in the rat vas deferens. Taken together, these results
demonstrate the importance of the amino acid residues in positions 17, 18, 19 and 20 for adequate CGP recognition and activation, and should be helpful toward the development of full selective analogues.

DIVERTICULAR DISEASE, INTESTINAL INFLAMMATION AND AUTOIMMUNE RESPONSES TO INTESTINAL MICROFLORA

631.4

PITUTARY ADENYLATE CYCLASE ACTIVATING PEPTIDE (PACAP) IS UP-REGULATED IN THE RAT DORSAL ROOT GANGLIA AFTER PERIPHERAL NERVE INJURIES. Y-Z. Zhang*, J. Hannibal, O. Zhao, N. Danielsen, K.
Møller, H. Møller, E. Eikblad, J. Fahrenkrug and E. Sandler.
Copenhagen, Denmark.

The effects of sciatic nerve injuries on the levels of PACAP and its
mRNA in the dorsal root ganglia (DRG) and the spinal cord were
investigated in male Sprague-Dawley rats. Animals were transected or
either transected or crushed in anesthetized rats. The sciatic nerve
on the right side was intact. The animals were killed 14 days
postoperatively. The spinal cord was dissected and processed for immunocyto-
chemistry, immunohistochemistry, in situ hybridization and northern
blot. Immunostaining of PACAP in the DRG was performed by using
of mainly small nerve cells in the DRG of the control side, confirming
our previous observations in normal DRG. PACAP was more intense in
the DRG of injured rats. The staining intensity of PACAP was seen in
many large cells. In the dorsal horn of the spinal cord, transection reduced PACAP intensity whereas crush injury revealed
no overt difference as compared to the control. The results from
northern blot and in situ hybridization supported the immunocyto-
chemical observations. The PACAP-mRNA in the DRG increased
significantly after nerve crush or transection injury.

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NEUROPEPTIDE-LIKE IMMUNOREACTIVITY IN BOVINE BRAIN CATHARTIN COATED VESICLES. W. L. Silva, L. Martinson, and N. Rosello. Department of Pharmacology, University of Central del Caribe, School of Medicine, Call Box 60-327, Bayamón, Puerto Rico 00621-6032. Cathatin coated vesicles (CCV) participate in the proteolytic maturation of neuropeptides (NP) in the trans Golgi network (TGN), and potentially in the internalization of neuropeptides and their receptors. In this study CCV from bovine brain gray matter were purified after chromatography over Sephacryl S-1000 columns. Fractons (13-25ml) were collected and analyzed for their adapter protein profile via SDS-PAGE, for their carbohydrate/peptide H (CPH) activity via radiodmyoassay, and for their NP (NP1, NP2, G-CCK, S-154) content by means of radioimmunoassays. The results revealed the presence of CPH and peptide-like immunoreactivités (NP-LP: NPY-LP, CCK-LP and SS-LI) associated with cathatin coated vesicles. The NP-LI and CPH were mainly associated with the ascending portions of the CCV peak suggesting that these molecules pertain to the expected larger diameter CCV subpopulation associated with the TGN, recycling and/or endocytic CCV. Analysis of the adapter protein profile of the material eluting from the Sephacyr S-1000 columns demonstrates a wide distribution of the Golgi-associated AP-1 (HA-1) molecules (AP47 and AP101) in relation to the CCV peak, spanning both the ascending and descending portions of the CCV peak. This adapter protein pattern is consistent with the inherent size microheterogeneity of TGN CCV, large diameter CCV of the regulated secretory pathway and smaller diameter CCV of the lysosomal pathway. Analysis of NP and NP processing enzymes in brain CCV will yield valuable information about the factors and conditions that influence the post-translational maturation and activation of NP. (This work was supported by NIH grants NS27259 and RR30105 awarded to WS).
SEROTONIN RECEPTORS: PHYSIOLOGY

632.1
Classically, 5-HT(2) receptors which modulate the firing activity of 5-HT neurons have also been suggested to control the somatodendritic release of 5-HT. We have recently shown that the somatodendritic 5-HT release is regulated independently of firing frequency via 5-HT(2) receptors (Neurosci. Abst. 127, 1999). The present study further assessed the regulation of somatodendritic release of 5-HT in a limited series of experiments, voltammetry studies using nafion-coated carbon fiber electrodes showed that the non-selective 5-HT agonist TPMP (0.5 mg/kg, iv) gradually reduced extracellular 5-hydroxyindoles in the dorsal raphe over a two-hour period. This effect was prevented by mianserin (2 mg/kg, iv), which blocks 5-HT(2) but not 5-HT(3) receptors, but not by the selective 5-HT(3) antagonist (1R)-WAY 103556, which only decreased the effect of 8-OH-DPAT (30 mg/kg, iv). The 5-HT(2) receptors are located on the somatodendritic 5-HT release in the raphe and may consequently play an important role in 5-HT release in widespread projection areas.

632.2
MODULATORY EFFECT OF 5-HYDROXYTRYPTAMINE (5-HT) ON CRAYFISH NEUROSECRETORY CELLS. F. Slenz, U. Garcia and H. Aran, Psychology*, Dept. Fisiología y Biotecnología, CINVESTAV, IPN México and *División de Estudios de Post-grado e Investigación, Facultad de Medicina, UNAM, México 11880. 5-hydroxytryptamine (5-HT) has been shown to influence the release of neurotransmitters from the crustacean eyestalk (see Fingerman and Neary, 1989). It is possible that one of the roles of 5-HT in the crustacean eyestalk is to modulate the release of hormones from the neurosecretory cells of the eyestalk. The present work, 5-HT was injected into the hemolymph of the crayfish eyestalk. The effects were measured using the most sensitive hormone of the shed gland, 11-deoxy corticosterone. 5-HT was administered to the eyestalk of the same population of both in situ, in isolated eyestalks and in cultured cells, taken from the same population. Two effects were found: a) a long-lasting (up to 30 min) dose dependent within a range of 10-4M, b) a short lasting, with a peak between 10-6M, close to the physiological range. This work was supported by CONACyT, grant number 3004-B1100.

632.3
In response to stressful stimuli, corticosterone (CT) production is increased by the hypothalamic-pituitary-adrenal (HPA) axis. One site of feedback regulation is the hippocampus, which contains high densities of both mineralocorticoid (MR) and glucocorticoid (GR) receptors. Basal plasma CT levels occupy the MR, high concentrations of CT activate the MR and the GR, both of which are implicated in the feedback control of the HPA. Activation of 5-HT(2) receptors decreases the slow afterhemorphalization (sAH) elicited by a train of action potentials or a calcium spike. To investigate if CT modifies the 5-HT(2) receptor mediated decrease of the sAH, intracellular recordings of the sAH following a calcium spike were obtained from the CA1 pyramidal cell layer of transversal hippocampal slices. Male Sprague-Dawley rats were adenalecetomized (ADX) 14 days prior to recording. One ADX group was chronically treated with a CT pellet (12.5 mg) to mimic basal plasma CT levels. Another ADX group was implanted with 300 mg CT to induce chronic stress. Data for continuous response curves were generated by addition of 5-HT in half log unit decrements (0.1-100 μM). Preliminary results suggest that the EC50 of the 5-HT induced decrease in sAH amplitude is higher in stressed animals than in ADX animals. When 1 nM CT was added to the superfusion medium of slices from ADX animals, the decrease in AHP amplitude was significantly more pronounced, indicating a short term modulatory role of the MR. The modulatory effects of CT on the 5-HT mediated response may underlie feedback control of the HPA and may influence pyramidal cell excitability. Supported by NS28512 and MH00880.

632.4
RECEPTOR RESERVE FOR 5-HT, RECEPTORS IN RAT BRAIN AS ASSESSED BY NEUROENDOCRINE RESPONSES TO 8-OH-DPAT STIMULATION. W. Pérez, O. Calvoa, C. Mir, L. de Bar and G. Batalona. Neuroscience Graduate Program and Dept. of Pharmacology, Loyola University of Chicago, Stritch School of Medicine, Maywood, IL 60153.
The present study investigates receptor reserve in female rats with respect to 5-HT1A mediated neuroendocrine responses. While previous studies have reported a large reserve for pre- and post-synaptic 5-HT1A receptors in male rats, 5-HT1A receptor reserve in female rats has yet to be determined. The stimulation of postsynaptic, hypothalamic 5-HT1A receptors with the full agonist 8-OH-DPAT, elicits an increase in several plasma hormones including ACTH and corticosterone. Therefore, neuroendocrine responses to 5-HT1A stimulation can be used as a functional end-point to assess receptor reserve. Female, adult rats (350-400 gms) were administered a single s.c. injection of vehicle (1:1 v/v EOB/H2O) or 1 mg/kg EEDQ (N-ethyl-N-octyl-2-(diethylamino) ethanol, 8-OH-DPAT). 24 hours post-treatment, rats were challenged with either vehicle (0.9% saline) or increasing doses (0.05, 0.2 and 0.5 mg/kg i.c.v.) of 8-OH-DPAT and sacrificed 30 mins later. There was a significant (p < 0.001) reduction (33%) in the Bmax of 8-OH-DPAT labeled hypothalamic 5-HT1A receptors in EEDQ-treated rats, with no alteration in KB. In non-EEDQ treated rats, 8-OH-DPAT elicited a significant (p < 0.001) dose-dependent elevations of plasma ACTH (4.0 fold at highest dose) and corticosterone (3.7 fold at highest dose). Following receptor inactivation by EEDQ, there was gg significant difference in the maximal ACTH (668.3 ± 148 vs 1025 ± 188 pmol/l) or corticosterone (434.3 ± 6.3 vs 50.3 ± 8.8 μg/dl) elevation elicited by 8-OH-DPAT. These data provide evidence for a large receptor reserve (at least 53%) for hypothalamic 5-HT1A receptors mediating the elevation of plasma ACTH and corticosterone levels in female rats. (Supported by DA07741 and Loyola University Potts Foundation).
632.5 MODULATION OF RAT NECORTICAL HIGH-VOLTAGE SPINDLE ACTIVITY BY SEROTONIN RECEPTOR SUBTYPE-SPECIFIC DRUGS. P. HARR, J. Sirvio, J. Kaski, E. Koskinen, M. Bökkilä, L. Yvesch, P. Rokkanen Sr. and P. Rokkanen Jr. Dept. of Neurology, University Hospital, SF-70211 Kuopio, Finland. Phone: 358-71-162048.

The present experiments investigated the role of serotonergic (5-hydroxytryptamine, 5-HT) receptors in the modulation of rat neocortical high-voltage spindle activity. We administered different 5-HT(3)/5-HT(7) receptor subtype specific drugs singly or in combination either systemically or via cannula that were implanted bilaterally to the medial thalamus, and measured neocortical high-voltage spindle activity of adult rats by IBM software. A mixed 5-HT(3)/5-HT(7) receptor antagonist, methysergide (p.), significantly increased neocortical high-voltage spindle activity, whereas another mixed 5-HT(3)/5-HT(7) receptor antagonist, methysergide (p.), had no effect. A 5-HT(3A)/5-HT(7) receptor agonist, (1,3-dimethyl-4-oxo-4-aminopropyl-2-aminopropane (DOI) (d.), significantly decreased neocortical high-voltage spindle activity, and systemic methysergide and specific 5-HT(3A)/5-HT(7) receptor antagonists ketanserin (k.) and rianismer (k.) blocked this effect. Further, intrathalamic injections of DOI dose-dependently decreased neocortical high-voltage spindle activity and systemic ketanserin completely blocked this effect. The results suggest that 1) the serotonergic system may via 5-HT(3A)/5-HT(7) receptors modulate rat thalamic neuronal activity as assessed by neocortical high-voltage spindle activity, 2) 5-HT(3A)/5-HT(7) receptor agonists may decrease neocortical high-voltage spindle activity, and 3) 5-HT(3A)/5-HT(7) receptor antagonists may block this effect. 5-HT(3A) may have a modulatory role to these receptors, in addition to a potentiating effect to the intrinsic properties of thalamic motor neurons. Therefore, 5-HT may function to facilitate the afferent input to thalamic motor neurons by potentiation of both NMDA and non-NMDA receptor-mediated synaptic processes.

632.6 EFFECTS OF 5-HT ON NMDA AND NON-NMDA RECEPTOR-MEDIATED RESPONSES IN TRIGEMINAL MOTOR NEURONS. C.R. Trueblood*, S.H. Chandler, M.S. Levine. Department of Physiological Science and Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

Previously, we demonstrated that 5-HT potentiated both the NMDA and non-NMDA mediated EPSP evoked by Mesencephalic V'Timulation in trigeminal motor neurons using an in vitro preparation of the brainstem from guinea pigs. To further examine the mechanism by which 5-HT facilitates excitatory amino acid responses in trigeminal motor neurons, we tested the effects of 5-20 μM bath application of 5-HT to NMDA (200μM) and AMPA (5mM) iontophoretic responses. In current clamp mode, 5-HT significantly potentiated both the NMDA and AMPA response in a dose-dependent manner. Selective agonists and antagonists were used to show that this potentiation resulted from activation of 5-HT(1c) receptors. Furthermore, using voltage clamp technique, 5-HT enhanced both the NMDA and AMPA current, suggesting that 5-HT has a direct modulatory role to these receptors, in addition to a potentiating effect to the intrinsic properties of trigeminal motor neurons. Therefore, 5-HT may function to facilitate the afferent input to trigeminal motor neurons by potentiation of both NMDA and non-NMDA receptor-mediated synaptic processes. Supported by NIH grants DE06193 and DE07212.


We have previously shown that local bilateral injection of hallucigenins d-LSD and (-)-DOB, but not non-hallucigenic congeners lianride and BL 3912A, into the medial prefrontal cortex (mPFC) elicited the head twiching response (HTR). This HTR was prevented by the selective 5-HT(2A) receptor antagonist, (-)-DOB, but 5-HT(3) receptors in the mPFC could play an important role in mediating the action of hallucigenins. Iontophoresis of low currents of (-)-DOB, and to a lesser extent d-LSD, markedly facilitated glutamate-induced activation of the first layer of mPFC cells. The aim of the present study was to determine whether pretreatment with mianserin or subacute treatment of d-LSD, which is known to down-regulate 5-HT uptake, also significantly reduce the drug-induced development desensitization of mPFC cells to d-LSD and (-)-DOB. Groups of rats were treated with d-LSD (2 mg/kg/day, i.p. for 5 days) and (-)-DOB (10 mg/kg, i.p. for 3 days) after the last injection of mianserin (10 mg/kg, i.p. 48 - 72 hr prior to experiment), and saline (1 ml/kg, i.p. either for 1 or 5 days), respectively. Compared to cells in the saline group, mPFC cells in rats pretreated with either d-LSD or mianserin became less sensitive to both drug treatments. Furthermore, in these drug pretreated rats, both d-LSD and (-)-DOB became less effective in stimulating the phosphoinositide hydrolysis in the mPFC. In conclusion, our results suggest that hallucinogen-induced tolerance and cross-tolerance may be accounted for, at least partly, by the functional desensitization of mPFC 5-HT(A) receptors at the cellular level. (Supported by USPHS grants MH-41440 and DA-07193).

632.9 FUNCTIONAL EVIDENCE FOR THE DIFFERENTIAL RESPONSIVENESS OF PRE- AND POSTSYNAPTIC 5-HT(A) RECEPTORS IN THE RAT BRAIN. P. Bier*, B. Seletti, C. Bouchard, F. Artigas and C. de Montigny, Neurobiological Psychiatry Unit, McGill Univ, Montreal, Canada H3A 1A1.

Several groups have documented the capacity of (p)indolol to antagonize the hypothermic effect of 5-HT(3A) agonists, including that of B-80-DPAT, whereas the increase in prolactin produced by the latter 5-HT(3A) agonist is blocked by similar doses of (p)indolol (Eur. J. Pharmacol. 146: 253, 1988). In a first series of experiments, the effectiveness of pindolol to block pencyramine-induced prolactin was examined by assessing the effect of dosal pencyramine on 5-HT(4) receptors in rats treated for 2 days with the 5-HT-uptake blocker paroxetine (10 mg/kg/day, s.c.) alone or together with (p)indolol (10 mg/kg/day, s.c.), both drugs being administered by an osmotic minipump. The firing rate of 5-HT neurons under chloral hydrate anesthesia was decreased by about 30% in both groups. However, the firing rate of 5-HT neurons was significantly increased by 5-HT in rats treated with pencyramine mg/kg/day of (p)indolol (which is the arrhythmic with 5-HT(4) affinity) for 2 days. In keeping with the latter observations, the suppressant effect of the 5-HT(4) receptor antagonist, 5-HT(4) receptor agonist on the firing of the pencyramine attenuated. In a second series, the prolactin increase produced by 8-OH-DPAT (100 μg/kg, s.c.) was also prevented by (p)indolol (15 mg/kg, i.p.) given 30 min prior to 8-OH-DPAT. The inhibition of prolactin levels by (p)indolol was increased if systemic applications of (p)indolol and 8-OH-DPAT on the firing rate of hippocampal CA1 pyramidal neurons was observed in rats treated with (p)indolol (15 μg/kg/day) for 5 days prior to 8-OH-DPAT. This increase activity was blocked by the 5-HT(A) receptor antagonist and the corresponding 5-HT(4) receptor. The block by the 5-HT(A) receptor antagonist modulating 5-HT neuronal firing activity and the post-synaptic 5-HT(A) receptor controlling prolactin release, but not the post-synaptic 5-HT(A) receptor coupled to K+ channels in the hippocampus.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
632.11
EFFECTS OF BUSPIRONE ON 5-HT TURNOVER IN DIABETIC AND NONDIABETIC RATS L. L. Bellush* and A.M. Wright. Dept. of Psychology, Ohio University, Athens, OH 45701.
Diabetic (D) rats have reduced 5-HT turnover throughout the brain. They also show attenuated hypothermia to the 5-HT1A agonist 8-OH-DPAT, as well as enhanced response to stress and greater anxiety, both of which could reflect altered 5-HT functioning. However, anxiolytic effects of the 5-HT1A partial agonist buspirone (BUSP) did not differ in D and nondiabetic (N) rats, and anxiolysis was greater after chronic BUSP. Here we measured 5-HT turnover in brainstem, midbrain, hippocampus and hypothalamus after single BUSP injections or after two weeks of daily treatment. BUSP had no effect on 5-HT turnover 20 min after acute treatment BUSP (0.1 mg/kg) in either D or N. BUSP did not affect light/dark emergence either, although D were more anxious than N, as well as having lower 5-HT turnover. BUSP (0.1, 0.6 and 1.2 mg/kg) reduced 5-HT turnover in all 4 regions of N rats 30 min after drug treatment. Here, the lower doses tended to reduce anxiety, while the high dose was anxiolytic. Assessment of 5-HT turnover after chronic BUSP 0.1 and 0.6 mg/kg in D and N is now being completed.

632.12
SEROTONINERGIC FIBERS ARE NOT DEVELOPED IN THE TRANSPLANTED HIPPOCAMPAL TISSUE OF THE S-100B RETARDED MUTANT MOUSE. S. Ueda*, P.M. Whitaker-Azima, E.C. Azimia and M. Kawata. Dept. of Anatomy, Kyoto Pref. Univ. Med. Kyoto 602, Japan; Dep. of Psychiatry, SUNY at Stony Brook, NY 11794, and Dep. of Biology, NYU, NY10003, USA. Our previous study (Ueda et al. 1994 Brain Res. 633,275-283) has demonstrated that the hippocampus of the homozygous of polyacyclury mutant mouse (Polyacyclury Nagoya, Pdn/Pdn) was markedly reduced in both S-100B positive astrocytes and serotoninergic fibers as compared to the heterozygote (Pdn+/-) and wild type (+/+). Since the Pdn/Pdn mice die within 2 days after birth, it is impossible to examine postnatal changes in development. To demonstrate the development changes of Pdn/Pdn hippocampal tissue, hippocampal pieces of neonatal Pdn/Pdn and +/- mice were transplanted into the right and left sides hippocampus of the same adult ICR mice, respectively, and immunocytochemistry was performed. Two weeks after transplantation, the S-100B positive astrocytes and a small number of serotoninergic fibers in Pdn/Pdn hippocampal tissue contained numerous GFAP positive astrocytes, while S-100B positive astrocytes and serotoninergic fibers were not observed. Two months after transplantation, GFAP and S-100B were expressed in the Pdn/Pdn hippocampal tissue similar to the +/- tissue. Serotoninergic fibers were distributed in the +/- tissue, whereas few serotoninergic fibers were observed in the Pdn/Pdn transplant tissue. On the contrary no difference was observed in the tyrosine hydroxylase positive fibers between Pdn/Pdn and +/- grafts.

632.13
Antidepressants drugs of different types increase extracellular 5-HT in the raphe nuclei of the midbrain. As a result, serotonin adrenoceptors are activated and terminal release decreases. Thus, the local infusion of 50 μM clonipramine (CT, a 5-HT uptake inhibitor) into the dorsal raphe nucleus (DRN) reduced in vivo release of 5-HT in striatum of the same animals by 47%. Pretreatment with (-) pindolol (15 mg/kg) or (-) sertralol (10 mg/kg) totally abolished the intra-DRN effects of CT, indicating that these reductions were mediated by 5-HT1A adrenoceptors. Lower doses of (-) pindolol (5 and 10 mg/kg, i.p.) induced a partial antagonism. In contrast, (-) WAY 100135 at a dose (10 mg/kg, i.p.) that antagonized the effects of 8-OH-DPAT (0.1 mg/kg, s.c.) on striatal release, was unable to prevent the CIT-induced reduction. These results indicate that the effects of 5-HT1A antagonists on serotoninetic 5-HT1A autoreceptors are antagonized by (-) pindolol and (-) sertralol, but not by (-) WAY 100135. The previous (3-3 days before) intra-DRN injection of pentsimate toxin also prevented the effects of CIT on striatal 5-HT release. This further supports the role of 5-HT1A autoreceptors in the attenuation of terminal 5-HT release elicited by CIT uptake inhibitors. In agreement, the combined treatment with (-) pindolol and 5-HT uptake inhibitors (paroxetine, citalopram, fluoxetine, fluvoxamine) increased terminal 5-HT release to a greater extent than the 5-HT uptake inhibitors alone (e.g., (-) pindolol + 1 μg CIT elevated striatal 5-HT release by +60% whereas the increase induced by 1 mg/kg CIT was +10%). Thus, blockade of serotoninetic 5-HT1A receptors may be a new way to potentiate the effects of low doses of 5-HT uptake inhibitors, in agreement with recent clinical data (Artigas et al., Arch. Gen. Psychiatry 51:248-251, 1994) (Supported by FIS grant 92/268).

632.14
5-HT2 RECEPTOR STIMULATION FACILITATES THE IN VIVO RELEASE OF ACETYLCHOLINE IN RAT FRONTAL CORTEX. H. Ladinsky*, S. Arnoldi, G. Rusli, G.B. Schilli and S. Congoli. Department of Biochemistry and Molecular Pharmacology, Boehringer Ingelheim Italia, Milan 20139 and Laboratory of Neuropharmacology, Istituto di Ricerche Farmacologiche "Mario Negri", Milan 20157 Italy.
The effect of the serotoninergic 5-HT2A receptor agonists BIMU 1 and BIMU 8 on the in vivo release of ACh in brain hemispheric regions of freely moving rats was investigated using the microdialysis technique. Both agonists, applied intracerebroventricularly, facilitated ACh release selectively in the frontal cortex at doses of 30-100 nmol. The agonists were ineffective in striatum, despite the high density of 5-HT2A receptors in this region. BIMU 1, 60 nmol i.c.v., produced a significant decrease in ACh release in the dorsal hippocampus. The facilitatory effect of BIMU 1 (40 nmol, i.c.v.) in frontal cortex was prevented by the highly selective and potent 5-HT2A receptor antagonists GR 125457 (10 and 20 nmol, i.c.v.) and GR 13380 (15 nmol, i.c.v.), which by themselves did not alter basal ACh release. The results provide evidence that serotonin facilities ACh release in frontal cortex through stimulation of 5-HT2A receptors that are not tonically activated. Assuming that ACh plays a role in cognitive processes, this facilitation supports the theory that 5-HT2A sites are important in the control of learning and memory. 5-HT2A receptor activation might thus offer a novel means of boosting central cholinergic function to overcome the cholinergic deficit in memory disorders. Financed in part by CNR, Rome, Convenzione Psicofarmacologia (S.C.).

632.15
Activation of central 5-HT1A receptors by the selective agonist 8-OH-DPAT decreases blood pressure (BP) and heart rate (HR). This study was to determine if the steroid hormone estradiol (E2) modulates the cardiovascular responses elicited by 8-OH-DPAT when administered into the lateral cerebral ventricle (l.c.v.). Experiments were performed in conscious, freely-moving rats implanted chronically with a guide cannula for i.c.v drug infusions and an arterial catheter to monitor BP and HR. 8-OH-DPAT (100 nmol, i.c.v) decreased BP and increased HR in male and ovariectomized female (OVX) rats. There was a tendency for a greater bradycardia in the OVX rats, but this did not attain statistical significance. The experiment was repeated to include OVX rats implanted subcutaneously with time-release pellets containing E2 (0.1 mg/21 day pellet). The BP response to 8-OH-DPAT was the same in all 3 groups: male, OVX and OVX+E2. By contrast, 8-OH-DPAT produced a greater bradycardia in the OVX+E2 group when compared to either male or OVX rats; OVX again tended to be greater than male. In the final experiment, graded doses of 8-OH-DPAT (3 - 100 nmol, i.c.v.) were administered to OVX and OVX+E2 rats. The hypotensive effect produced by 8-OH-DPAT was not altered by hormone treatment, but the bradycardia was greater across doses in the E2-treated rats. In summary, the cardiovascular responses elicited by 8-OH-DPAT were greater in both males and OVX rats. However, E2 enhanced the bradycardia, but not the hypotension, elicited by 8-OH-DPAT, suggesting that estrogen modulates some, but not all, functional response produced by 5-HT1A receptor activation. (Supported by NIH Grant NS 29765)
633.2
Presently, a new radioiodinated agonist, [1]-[35]OH-DPAT, for 5-HTA receptors, was reported (Kung et al., Neuroscience Abs, 1993). In developing antagonists for 5-HTA receptors, we have selected several intrinsic agonists of the phenylpiperazine (Zhang et al., J. Med. Chem., in press). Among them, MPPI (4-[2-methoxy-phenyl]-1-[2-n-propyl]-p-iodobenzamido-jethyl-piperazine) displayed high efficacy for 5-HTA receptors (5-HTA) in rat hypothalamic homogenates. Low to moderate binding affinity of MPPI to 5-HTA receptors was observed with Ki values of 15, 38, 170, and 270 nM, respectively. Saturation binding studies in rat hippocampus homogenates revealed that [125I]MPPI bound to a single population of affinity sites with a Kd of 0.18 nM (Kd = 0.4 nM, Bmax = 120 fmol/mg) in rat hypothalamus. Thus, in [125I]MPPI was 30-40% higher than that with [125I]OH-DPAT. Guaneryl nucleotides did not affect the specific binding of [125I]MPPI, but significantly inhibited the specific binding of [125I]OH-DPAT. In addition, Me2+ ion showed an inhibitory effect on [125I]mppi binding, in contrast to the stimulatory effect observed for the agonist ligand, 8-OH-DPAT.

633.4
The lack of selectivity of 5-HTA receptor antagonists has hampered study of the functional role of these receptors. A candidate for such an antagonist, p-MPPI, is a structural analog of WAY-100635 with high affinity for 5-HTA receptors (Kd = 10 nM). p-MPPI was tested for its ability to antagonize the effects of the 5-HTA receptor agonist 8-OH-DPAT on two responses mediated by postsynaptic 5-HTA receptors, the 5-HT syndrome and hypothermia. Male Sprague-Dawley rats were pretreated with p-MPPI (3,30 mg/kg, s.c.) 15 min prior to 8-OH-DPAT injection (1,2 mg/kg in the hypothermia and 5-HT syndrome studies, respectively). p-MPPI dose-dependently prevented p-MPPI-induced hypothermia and 8-OH-DPAT-induced hypothermia (production of four out of the six components behaviors) induced by 8-OH-DPAT. However, p-MPPI did not prevent two of these behaviors, flat body posture and hindlimb abduction. p-MPPI did not produce any of the 5-HT syndrome effects when given alone. In rats pretreated with reserpine (1 mg/kg, 18 hours), p-MPPI (30 mg/kg) also blocked the 5-HT syndrome without producing any symptoms when given alone. Thus, p-MPPI antagonized the hypothermic effect produced by 8-OH-DPAT, whereas p-MPPI did not antagonize the hypothermic effect produced by reserpine. These results demonstrate that p-MPPI antagonizes 5-HTA receptors with a partial agonist activity in combination with 8-OH-DPAT, on extracellular levels of 5-HT using in vivo microdialysis. This research was supported by USPHS grants MH 14654, MH 36202 and MH 48125.
633.7


Molecular cloning has demonstrated that the 5-HT1D subtype is comprised of two closely related subtypes termed 5-HT1Dα and 5-HT1Dβ. These receptors display a high transmembrane homology (~75%) which contribute to their similar pharmacological profiles. The classical 5-HT2 agonists, ketanserin and ritanserin, displayed moderate affinity (K<sub>i</sub> 50-75 nM) and marked selectivity (K<sub>i</sub> 5-HT2/5-HT2) for the cloned human 5-HT2D subtype relative to the 5-HT1D receptors. In contrast, the nonselective 5-HT2 receptor antagonist, methiothepin, exhibited similar affinities (K<sub>i</sub> 10-20 nM) for both 5-HT1D subtypes. These three compounds were evaluated for their ability to antagonize sumatriptan-induced inhibition of forskolin-stimulated cAMP accumulation in cell lines expressing either 5-HT<sub>1D</sub> or 5-HT<sub>1D</sub> gene. All three compounds behaved as competitive antagonists devoid of intrinsic activity in the functional assays. The apparent K<sub>i</sub> values measured in the functional assays closely matched their K<sub>i</sub> values obtained in binding assays.

633.9


Binding sites displaying high (5-HT<sub>1D</sub>) and low (5-HT<sub>1D</sub>) affinity for 5-carboxyamidotryptamine have been described using [3H]-5-HT and serotonin-selective 5-HT<sub>1D</sub> and 5-HT<sub>2</sub> receptors. Utilizing [3H]-CP-101,606 (0.5 nM) as the radioligand eliminated binding to the 5-HT<sub>1D</sub> sites. However, potent 5-HT<sub>1D</sub> agonist compounds such as sumatriptan and BMS-181101 (a novel antidepressant candidate) continue to produce a regionally distinct biphasic displacement (K<sub>i</sub>20 nM, 2 nM and K<sub>i</sub>10 nM, 10 μM, sumatriptan and BMS-181101, respectively), suggesting a heterogeneous population of 5-HT<sub>1D</sub> receptors radiolabeled with [3H]-CP-101,606 in certain mammalian brain regions. In bovine substantia nigra, both sumatriptan and BMS-181101 produced monophasic displacement of [3H]-CP-101,606 binding. Therefore, this tissue was utilized initially to characterize the agonist profile of PHBMS-181101 to 5-HT<sub>1D</sub> binding sites. In bovine substantia nigra membrane homogenates, [3H]-PHBMS-181101 binding was rapid, reversible and saturable, displaying high affinity (K<sub>i</sub> 1 nM) and low non-specific binding. Pharmacologically, affinity values revealed the following rank order of potency: 5-CT<sub>D</sub>5-HT<sub>L</sub>S=5-DHE- sumatriptan methiothepin oxypertin triptan [CP-101,606]. CP-93,129, mesulergine, buspirone and sironere all displayed negligible affinity. Gpp(NH)p induced a concentration-dependent decrease in high affinity [3H]-PHBMS-181101 binding, suggesting an agonist interaction with the binding site.

These data indicate that functional inhibition of 5-HT<sub>1D</sub> receptors may be involved in the antidepressant effect of atypical neuroleptics.

633.11

GR127935: IN VITRO AND IN VIVO CHARACTERIZATION OF A PUTATIVE 5-HT1D AGONIST ANTAGONIST: F. D. Tingley, P. W. Smith, H. R. Howard, and D. W. Schultz, Central Research Division, Pfizer Inc., Groton, CT 86340.

Functional studies of 5-HT<sub>1D</sub> receptors have been hampered by the limited receptor selectivity of available antagonists (e.g. methiothepin). Recently, it was reported that the bipharyl carboxamide GR127935 (5-HT<sub>1D</sub>) is a high affinity for the cloned human 5-HT<sub>1D</sub> and 5-HT<sub>1D</sub> receptors, displays >100-fold selectivity vs other receptor subtypes, and behaves as an agonist based on its reversal of contraluminal rotation in guinea pigs caused by intraintestinal administration of a 5-HT<sub>1D</sub> agonist (Br J Pharmacol. 110:97-199, 1993).

We have further characterized the functional activity of GR127935 at 5-HT<sub>1D</sub> receptor sites in guinea pig brain tissue using an extracellular calcium activity assay in substantia nigra and 2) examining changes in 5-HT turnover in guinea pig hypothalami following intraperitoneal administration. Adenylate cyclase studies revealed that GR127935 is a full agonist at 5-HT<sub>1A</sub> receptors and a partial agonist at 5-HT<sub>1D</sub> receptors (IC<sub>50</sub> 10<sup>-7</sup> M). The effect on 5-HT turnover in guinea pig hypothalami following intraperitoneal administration was characterized by a significant increase in 5-HT<sub>1A</sub> receptors, 2 nM at 5-HT<sub>1D</sub> receptors are consistent with the report receptor selectivity profile of GR127935.

In vivo, s.c. treatment with GR127935 reversed the decrease in 5-HT turnover (5-HIAA/5-HT ratio) caused by the 5-HT<sub>1D</sub> agonist agonist CP-135,807 (see Schmidt et al., Soc. Neurosci. Abstr., 1994). When administered alone, GR127935 dose-dependently increased 5-HT turnover. The 5-HT<sub>1D</sub> agonist agonist is a potent 5-HT<sub>1D</sub> antagonist of 5-HT<sub>1A</sub> terminal autoceptor.

633.12


The use of sumatriptan to study central 5-HT<sub>1D</sub> receptors is limited by its poor brain penetration. Here we describe the biochemical and pharmacological properties of CP-135,807 (3-[pyrrolidin-2-ylmethy1]indole), a novel 5-HT<sub>1D</sub> agonist with improved potency and CNS penetration.

1) CP-135,807 (3-[pyrrolidin-2-ylmethy1]indole) binds with high affinity (K<sub>i</sub> 1.3 nM) to brain 5-HT<sub>1D</sub> receptors (Kee et al., Soc. Neurosci. Abstr., 1994). Like sumatriptan, CP-135,807 behaves as an agonist by inhibiting the cyclic AMP activity of 5-HT<sub>1D</sub> (guinea pig substantia nigra, E<sub>50</sub> 0.1 nM) and 5-HT<sub>1A</sub> receptors (guinea pig hippocampus, E<sub>50</sub> 41 nM). Sumatriptan has a similar receptor selectivity profile, h<sub>5-HT<sub>1A</sub></sub> with much less potent (E<sub>50</sub> 0.7 nM) activity.

Using an ex vivo binding, it was determined that CP-135,807 readily crosses the blood-brain barrier, while sumatriptan was undetectable in brain after a subcutaneous dose of 100 μM. This is consistent with a compound that activates 5-HT<sub>1D</sub> terminal autoceptors, s.c. CP-135,807 dose-dependently inhibited 5-HT turnover in guinea pig brain, decreasing 5-HIAA/5-HT ratios by up to 50%. Consistent with this mechanism of action, in vivo studies demonstrated that perfusions with 0.2-10 μM CP-135,807 through a microdialysis probe in the hypothalamus caused a dose-dependent decrease in extracellular 5-HT. The centrally-mediated effect of CP-135,807 on 5-HT turnover is consistent with its ability to cause hyperthermia in guinea pigs (Seymour et al., Soc. Neurosci. Abstr., 1994).
633.13
CP-135,807, A NOVEL 5-HT1D AGONIST INDUCES HYPOThERMIA IN THE GUINEA PIG. D.A. Seymour*, D.W. Schulz, F.D. Tingley and J.E. Macor. Departments of Neuroscience and Medicinal Chemistry, Central Research Division, Pfizer Inc, Groton, CT 06340.

Central serotonergic systems have long been implicated in thermoregulation. The 5-pyrrolidin-2-R-ylmethyl)indole, CP-135,807, has recently been characterized as a novel 5-HT1D agonist in vitro with a high level of selectivity vs. 5-HT1A receptors as measured by inhibition of adenylyl cyclase activity (Schmidt et al., Soc. Neurosci. Abstracts, 1994). To investigate the functional implications of CP-135,807's effect in vivo we determined that CP-135,807 lowers body temperature in cpd, a known to have central 5-HT1D receptors. Rectal temperatures were measured in awake rats (60-120 and 240 min after either s.c. or p.o. administration. It was found that CP-135,807 profoundly and dose-dependently lowers body temperature after both routes of administration, with greater potency obtained after the s.c. route. This effect appears to be centrally mediated since sumatriptan and CP-123,803, an analog with a similar binding profile that does not reach the CNS, failed to produce this response. In addition, the putative 5-HT1D antagonist, GR-127935, antagonizes this effect when given 60 min prior to agonist administration. These data suggest that 5-HT1D receptors play a thermoregulatory role in guinea pig, and that this response may be useful in the characterization of drugs that act at 5-HT1D receptors.

633.14
NOVEL TRYPATINE DERIVATIVES WITH A 5-(3-NITRO-2- PYRIDYLAMINO) MOIETY BIND PREFERENTIALLY TO 5-HT1D RECEPTORS. B.K. Koe*; L.A. Lebel, C.B. Fox, and J.E. Macor. Central Research Division, Pfizer Inc, Groton, CT 06340.

The recent introduction of serotoninergic drugs (5-HT reuptake inhibitors as antidepressants; 5-HT1A agonists as anxiolitics; a 5-HT1D antagonist, CP-123,803, as an antimalarial agent) as well as the chemical description of new and distinct 5-HT receptors have spurred interest in finding selective agonists for these receptors. One approach is based on modifying the serotonin molecule by introducing novel substituents at the indole C5 and C9 positions. Examples from our laboratories include a selective 5-HT1D agonist, CP-63,129, a potent and 5-HT1A, 5-HT1B, and 5-HT1D receptors and the DA transporter, CP-110,330 (Koe et al., 1992; Nowakowski et al., 1993). We now report that the 5-(3-nitro-2-pyridylamino) moiety (R) confers 5-HT1D binding selectivity to a novel pyridylamine (3-M).
634.5 EFFECTS OF PSYCHOSOCIAL STRESS ON 5HT1A RECEPTORS IN THE BRAIN. G. Fliege* and E. Fuchs. German Primate Center, 37077 Göttingen.

Normal serotonergic neurotransmission in the brain requires normal numbers of receptors. Perturbing conditions such as sustained psychosocial stress (PSS) may disturb the functional balance of the transmitter systems. In the present study, we investigated the effects of PSS on 5HT1A receptors in the brains of male Wistar rats (* to a group of animals that underwent 24 hours of PSS followed by 24 hours of social isolation. All rats were killed 6 hours after the end of the PSS. We determined the number of 5HT1A receptors in various brain regions using high affinity 5HT1A receptor-specific antagonist binding. Because 5HT1A receptors are involved in the regulation of social behavior, one might expect that PSS would influence the 5HT1A receptor expression. However, our findings revealed no significant differences in the number of 5HT1A receptors in brain regions when compared to sham-operated controls. Our data do not support the hypothesis that PSS influences 5HT1A receptor expression. Further investigations of other subtypes of 5HT receptors and additional behavioral parameters are needed in order to clarify the role of serotonergic neurotransmission in stress-related phenomena.

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634.6 EFFECTS OF 5-HT AND 8-OH-DPAT ON SPONTANEOUS OPEN-FIELD MOTOR ACTIVITY AFTER LOCAL APPLICATION INTO THE SUBSTANTIA NIGRA PARS RETICULATA (SNr) AND VENTRAL TEGMENTAL AREA (VTA) IN THE RAT. V. Hillesgård* and S. Ahlenius. Department of Behavioral Pharmacology, Astra Arcus AB, S-151 85 Södertälje, Sweden.

The 5-HT1A receptor agonist 8-OH-DPAT (0.5 μg), or 5-HT (0.4 μg), was infused into the SNr or into the VTA in awake, gently hand-restrained male Wistar rats (* to a group of animals that underwent 24 hours of PSS followed by 24 hours of social isolation. All rats were killed 6 hours after the end of the PSS. We determined the number of 5HT1A receptors in various brain regions using high affinity 5HT1A receptor-specific antagonist binding. Because 5HT1A receptors are involved in the regulation of social behavior, one might expect that PSS would influence the 5HT1A receptor expression. However, our findings revealed no significant differences in the number of 5HT1A receptors in brain regions when compared to sham-operated controls. Our data do not support the hypothesis that PSS influences 5HT1A receptor expression. Further investigations of other subtypes of 5HT receptors and additional behavioral parameters are needed in order to clarify the role of serotonergic neurotransmission in stress-related phenomena.

The present study evaluated the contribution of serotonin (5-HT2A/2C) receptors to the in vivo actions of clozapine and several, putative atypical antipsychotics. In a drug discrimination paradigm employing animals trained to discriminate the 5-HT2A/2C agonist, DOI (0.63 mg/kg, i.p., from saline, the discriminative stimulus (DS) effects of DOI were dose-dependently and completely blocked by the 5-HT2A antagonist, ketanserin (10 mg/kg, i.p.), the selective 5-HT2A receptor antagonist, M101,907 (0.001, 0.003, 0.01 mg/kg, i.p.). Like M101, 907, both clozapine and two putative, atypical antipsychotics possessing marked affinity at 5-HT2A receptors, risperidone and sertindole, blocked the DS effects of DOI (0.05, 0.03 and 0.32, respectively). Both compounds abolished the head-twitches (HTW) evoked by DOI (2.5 mg/kg, s.c.). DS: 0.28, 1.21, 0.02, 0.06, 0.005, 0.04, 0.025 and 1.0, respectively. The dopamine D2 antagonist, haloperidol, failed to block the DS effects of DOI (dose-range tested: 0.01 - 0.16), but abolished DOI-induced HTWs (DS: 0.07). In conclusion, these data demonstrate the role of 5-HT2A receptors in mediating both the effects of the HTWs and the DS assessed by DOI, though the latter response is also modulated by D2 receptors indicating the superior selectivity of the drug discrimination paradigm. The marked activity of clozapine, risperidone, sertindole and MDL 101,907 in these models support the concept that 5-HT2A receptor antagonism contributes to the in vivo actions of atypical antipsychotics.


Antisense (A) phosphorothioate oligonucleotide specific to 5-HT2A receptor mRNA were administered in media to cultured C6 glioma cells and ICV (intracerebroventricular) to rats. In C6 cells, significant decreases in 5-HT2A receptor density and gene expression were evident after 3d treatment with A but not control (C) sequences (20-200 nM). In male Long-Evans rats, treatment with A (50 µg ICV twice daily x 4: tests were 24 h after final dose), but not C, resulted in a significant decrease in 5-HT2A receptor mediated headshaking induced by the 5-HT2A agonist DOI ((2R,1S,2S)-1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane hydrochloride); conversely, a significant increase in DOI-induced, 5-HT2A-mediated, skin scratching behavior was observed. A significant anxiolytic-like effect was observed in A but not in C-treated rats tested in the elevated plus-maze. Autoradiographic and saturation analysis of 5-HT2A and 5-HT3b binding from the rats in the behavioral studies indicated significant, 50-72% decreases in 5-HT2A binding, and no change in 5-HT3b binding. These results demonstrate that 5-HT2A receptor expression and function can be selectively attenuated using A sequences, and suggest that the 5-HT2A receptor inhibits agonist-induced 5-HT2A receptor-mediated responses. (Supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development.)

634.13 TREATMENT WITH 5-HT2A/2C RECEPTOR ANTAGONIST DISRUPTS INITIAL DEVELOPMENT OF SPATIAL NAVIGATION STRATEGIES IN RATS. M. Riekkinen* J. Sirviö and P. Riekkinen, Dept. of Neurology, Univ. of Kuopio, P.O.B. 1627, FIN-70211, Kuopio, Finland. Fax: 358-71-162048.

The present study investigates the effects of 5-HT2A/2C receptor stimulation on spatial and cue navigation, and visual discrimination. Daily s.c. injection of DOI (5,5-dimethoxy-4-iodo-phenyl)-2-amino-propane (DOI) at 3 mg/kg, but not at 1 mg/kg, impaired initial development of water maze (WM) spatial navigation strategies (hidden platform), but had no effect on the development of non-spatial navigation strategies (visible platform). Pre-training injections of ketanserin (0.1, 0.4 and 2 mg/kg, i.p.), a 5-HT2/2C receptor antagonist, had no effect on WM performance, but blocked the impairing effect of DOI 3 mg/kg on WM spatial navigation. Spatial reference and working memory were not disrupted by pre-training DOI (1 and 3 mg/kg) treatment if the rats had learned the reference memory rule before drug treatment. 5-HT2A/2C receptor stimulation did not impair consolidation as post-training DOI at 1 and 3 mg/kg had no effect on the development of spatial navigation strategies. Visual discrimination learning was not impaired by pre-training DOI 1 and 3 mg/kg. The present results suggest that 5-HT2A/2C receptor stimulation impairs initial development of spatial navigation strategies in rats.

635.1 IN VIVO STUDIES OF 5-HT2 RECEPTORS IN MONKEYS USING F-18-ALATANERIN. C.A. Mathis*, Y. Cho, N.R. Simpson, M.A. Minton, PET Faculty, University of Pittsburgh Medical Center, Pittsburgh, PA 15213.

Alatinerin is a closely related structural analogue of ketanserin and has a high in vitro affinity and selectivity for 5-HT2 receptors. We examined the in vivo properties of this radiogand in monkeys as a candidate for imaging brain 5-HT2 receptors using positron emission tomography (PET). Three rhesus monkeys received 10 mCi of high specific activity (1100-2500 Ci/mmol) F-18-alatinerin. In addition, one monkey received a second 10 mCi injection of low specific activity (13 Ci/mmol) F-18-alatinerin. Twenty scans of increasing duration were acquired over a 12.0 min time period. Arterial blood samples were obtained and the plasma radioactivity was assayed for the presence of metabolized metabolites. In two monkeys, ketanserin and alatinerin (1 mg/kg) were injected 2 h after the injection of F-18-alatinerin to displace specifically bound radioactivity. At times up to 37 min, selective ketanserin radioactivity was observed in cortical areas while cerebellum, caudate, and thalamus had low radioactivity levels. The ratio of frontal cortex to cerebellum was 2.5±0.3 in the high specific activity studies and decreased to 1.5 at 2 hours after the tracer injection in the low specific activity study. A two compartment model data yielded kinetic rate constants K1, k2, and distribution volume (DV). Estimates of DV, which is proportional to brain volume, were 3.4±0.1 for frontal cortex, 2.1±0.5 for caudate, and 1.7±0.5 for the cerebellum. The DV's varied by region. The alatinerin data yields consistent with the known distribution of 5-HT2 receptors. Both ketanserin and alatinerin exhibited a distribution of 5-HT2 receptor distribution in cortical areas with displacement half-times of 23 and 37 min, respectively. Unmetabolized alatinerin comprised 50±1% of the total plasma activity at 2 h post injection. These results indicate that F-18-alatinerin is a useful agent to image and quantitative 5-HT2 receptors in vivo using PET.

635.2 LOCALIZATION OF 5-HT2 RECEPTOR mRNA IN NEURONS OF THE HUMAN BRAINSTEM. M.C. Austin*, J.A. Winkel and J.J. Marks, Labs of Neuropharmacology, Dept. of Psychiatry, Univ. of Pittsburgh. Pittsburgh, PA 15213.

A previous report autoradiography study, using [3H]LSD at a ligand, reported the anatomical localization of serotonin receptors in the human brainstem (Palacios et al., 1983). This study found that the highest levels of binding occurred predominantly in the raphe nuclei, thus revealing the localization of serotonin receptors of the 5-HT-like subtype. Given the recent cloning of the human 5-HT2 receptor (Chen et al., 1992), we examined the distribution of neurons expressing 5-HT2 receptor mRNA in the human brainstem using in situ hybridization histochemistry (ISHH). Postmortem human brainstem tissue was fixed at 4°C in 4% paraformaldehyde. After the level of the hypoglossal nucleus, the dorsal motor nucleus of vagus and the gigantoacellular reticular nucleus all contained abundant levels of 5-HT2 mRNA. In the periaqueductal region, neurons containing cells that were also 5-HT2 mRNA-positive were also found in the oral pontine nucleus. A large population of neurons expressing 5-HT2 receptor mRNA was found in the diffuse core of the pedunculopontine tegmental nucleus, and several 5-HT2 mRNA-positive neurons were located in the caudal portion of the substantia nigra. These findings provide important information regarding the localization of 5-HT2 receptors and the relevant nuclei in the human brainstem that can serve as a potential serotonin immunization. (Supported by MH30915, MH64765)
SEROtonin Receptors: Localization

635.3


The anatomic distribution of 5-HT receptors in rat brain and spinal cord has been defined by radioiodinated binding studies and quantitative autoradiography using the specific, high affinity radioligand, [3H]-8-OH-DPAT. We are currently using a synthetic antipeptide antibody to characterize the localization of the 5-HT3 receptor protein in the spinal cord. This polyclonal antibody was raised in rabbit against amino acid sequence 170-186, a transmembrane region of the 5-HT3 receptor that is structurally related to the ligand binding domain of other G-protein coupled receptors. In the spinal cords of rats, cats and macaques we observed the highest density of 5-HT3-R in the superficial layers of the dorsal horn on primary afferent sensory neurons and in nerve fibers running along the central canal. This pattern of 5-HT3 receptor-[3H]-8-OH-DPAT binding corresponds precisely to the autoradiographic localization of [3H]-8-OH-DPAT described in the rat spinal cord. The most notable pattern of 5-HT3 receptor-[3H]-8-OH-DPAT binding we observed was the axon hillock of motoneurons throughout the ventral horn. This novel site of 5-HT3 receptor localization is not detected with [3H]-8-OH-DPAT, suggesting possible heterogeneity of 5-HT3 receptor signal transduction mechanisms. A dense population of 5-HT3 receptors on the motoneuron axon hillock indicates an important role in the regulation of motor output. Species differences in 5-HT3 receptor-[3H]-8-OH-DPAT binding were noted, for example, in concentration of fiber labeling in the dorsal horn, the pattern of 5-HT3-R around the central canal, and the intense somatodendritic labeling of spinal serotoninergic (raphe) cells in the primate cervical spinal cord. (Supported by NIA # PO1 AG10208)

635.4

DISTRIBUTION OF 5-HT1A RECEPTORS IN THE PRIMATE BRAINSTEM J.P. Gannon*, N.M. Kheif, and E.C. Armita. Dept of Otolaryngology, Mt Sinai Sch Med, NY NY10022 Dept Biology, New York University NY NY10030. We used an 5-HT1A receptor antipeptide (aa170-186) antibody to investigate the distribution of this serotonin receptor subtype in the brainstem of the Old World monkeys Macaca mulatta and Macaca nemestrina. Three adult monkeys were fixed by transcardiac perfusion, then 40μm brainstem sections incubated in primary antibody 1:2000 for 72 hours at 0°C followed by visualization of secondary antibody.

Three main types of cell labeling patterns were apparent throughout the brainstem: 1) on neurons of both the serotonergic raphe nuclei and the noradrenergic locus coeruleus, dense labeling was distributed diffusely throughout the soma and dendritic tree. Similarly labeled large neurons were scattered throughout the reticular formation; 2) Most neurons in the brainstem motor nuclei showed a discrete label localized specifically to the axon hillock. This axon hillock pattern was also seen throughout the brainstem on some neurons of both sensory and reticular groups. Sub-populations of vestibular nuclear neurons were also labelled; 3) A diffuse general label was present on astrocyes throughout the brainstem. The somatodendritic label we observed may represent receptor populations acting as autoreceptors. The dense axon hillock label may represent populations of 5-HT1A receptors involved in temporally dynamic regulation of motor output at this critical cell location. During primate evolution, basal mammalian motor systems changed and to survive the more sophisticated communicative repertoire present in primates. These evolutionary advances in brain function involved incorporation of the 5-HT1A receptor as a key player. (Work supported in part by NIA Grant PO1 AG10208)

635.5


The present study examined the comparative distribution of 5-HT1A receptor mRNA and 5-HT1A binding in human hippocampus using in situ hybridization with a specific cRNA probe and receptor autoradiography with the selective ligand [3H]-8-OH-DPAT. In agreement with previous binding studies, the highest levels of 5-HT1A binding in the human hippocampus were observed in the CA1 field and in the subiculum. High levels of binding were also observed in the granular and molecular layers of the dentate gyrus. Intermediate levels were present within the pyramidal and molecular layers of CA2 and CA3 subfields. In contrast to 5-HT1A binding, the highest concentration of 5-HT1A mRNA expression was observed in the granular layer of the dentate gyrus. Similarly, the positive signal was also observed in the molecular layer of CA1, CA2 and CA3 subfields, although the mRNA levels were lower than in the dentate gyrus. No 5HT1A mRNA was detected in the stratum oriens, radiatum or moleculare. The subiculum and the external granule layer of the parahippocampal gyrus also expressed 5HT1A mRNA at concentrations similar to the CA subfields. In conclusion, there was a concordance between the areas expressing 5-HT1A mRNA with the areas showing specific 5-HT1A binding in human hippocampus. However, there are differences in distribution and concentration between mRNA and receptor sites within specific anatomical regions. These differences are probably due to the cytoarchitectural distribution of the neuronal cell bodies, where the 5-HT1A mRNA is transcribed, versus the apical and basal dendrites, where the 5HT1A receptors are localized. Supported in part by a grant from the American Suicide Foundation.

635.7


The evidence to date for the presence of serotonin receptors on astrocytes in vitro has mainly been dependent on ligand binding and second messenger assays. We have demonstrated the expression of serotonin receptor subtype mRNAs using in situ hybridisation. CDNA clones of 5-HT1A, 5-HT1B and 5-HT1D, subcloned into suitable vectors were used to generate specific 35S-labelled riboprobes. These were hybridised to neonatal rat cortical astrocyte cultures. Results show that the astrocytes in vitro express mRNAs for the above three receptor subtypes. Data from binding experiments and second messenger assays complement the in situ hybridisation results, showing that the astrocytes express the receptor protein and that it is functional.

We have also addressed the question of serotonin receptor subtype expression on astrocytes in adult rat brain sections using a combination of in situ hybridisation of receptor subtype specific cRNAs and immunohistochemical localisation of the astrocyte specific marker GFAP. We found little evidence of co-localisation of receptor specific cRNAs and immunohistochemical localisation of the astrocyte specific marker GFAP. We found little evidence of co-localisation of receptor specific cRNAs and immunohistochemical localisation of the astrocyte specific marker GFAP. We found little evidence of co-localisation of receptor specific cRNAs and immunohistochemical localisation of the astrocyte specific marker GFAP. We found little evidence of co-localisation of receptor specific cRNAs and immunohistochemical localisation.
636.5  
LOCALIZATION OF 5-HT6 RECEPTOR mRNA IN THE RAT BRAIN BY IN SITU HYBRIDIZATION HISTOCHEMISTRY. B. P. Ward1, M. W. Hamblin2, B. J. Hoffman3, J. E. Latchev4, D. R. Sibley4, and D. M. Donsi5. 1Dept. of Pharmacology, Dept. of Psychiatry and Behavioral Sciences, Univ. of Washington, Seattle, WA 98195; 2GRECC, Seattle VAMC, Seattle, WA 98101; 3Molecular Neuropharmacology Section, Experimental Therapeutics Branch, NIDA, NIH, Bethesda, MD 20892; 4Laboratory of Cell Biology, NIH, N.IH Bethesda, MD 20892.

The serotonin receptor subtype 5-HT6, which raises intracellular cAMP via stimulatory G proteins, has been recently characterized. To determine the CNS distribution of 5-HT6 mRNA, in situ hybridization was performed in coronal sections of rat brain. An [35S] labeled riboprobe, complementary to the 5-HT6 mRNA coding region was used. In situ hybridization was performed on brain sections using the recombinant full length cDNA sequence as a probe. The 5-HT6 receptor was found to be expressed in the neocortex, hippocampus, amygdala, and the hypothalamus. The distribution of 5-HT6 mRNA was characterized in different brain regions and was found to be highest in the hippocampus, amygdala, and hypothalamus. This distribution pattern suggests that the 5-HT6 receptor plays a role in the regulation of neuronal activity in these regions.

636.1  
PHARMACOLOGICAL CHARACTERIZATION OF THE EFFECT OF EXTRACELLULAR ADENOSINE 5'-TRIPHOSPHATE ON THE CONCENTRATION OF FREE CYTOPLASMIC CALCIUM IN RAT PHEOCHROMOCYTOMA PC12 CELLS. E. Adamiec1 and G. Kosti2. 1Dept. of Anesthesia, Massachusetts General Hospital, Boston, MA 02114.

In PC12 cells, ATP causes substantial elevation of 
Ca2++](free buffer] of approximately 10% of the total calcium in intracellular stores to the total [Ca2++](free buffer] rise. The majority of ATP-induced [Ca2++] is caused by Ca2+ influx. The [Ca2++] increase is positively correlated with the concentration of free, uncomplexed ATP (ATP↑) suggesting that ATP↑ is the agonist at the ATP receptor in this cell type. Administration of UTP also increased [Ca2++] by increasing extracellular free Ca2+ to 32 mM, ERCp (30 μM). This elevation also consisted of a combination of Ca2+ influx and Ca2+ release from internal stores. The ATP↑ response to ATP and UTP share a common receptor (P2Y). The Ca2+ values for the ATP- and UTP-induced ATP↑ were not significantly different. ATP↑ caused a much larger maximal [Ca2++] increase, indicating that ATP acts as a full agonist at P2Y receptor in PC12 cells.

636.3  

We reported that ATP stimulates P2 purinoceptors, activates Ca-permeable cation channels, stimulates Ca influx and dopamine (DA) release in PC12 cells (a review in NIPS, April, 1992). We have also reported that endogenous transmitters, dopamine (Eur. J. Pharmacol., 215, 321-324, 1992; Pflügers Arch., 422, 458-464, 1993), 5-iodo-ADP, and adenosine (Br.J.Pharmacol., in press, 1994; Eur.J.pharmacol., in press, 1994) regulate the ATP-evoked responses. We report here that zinc also regulates ATP-evoked currents and DA release from PC12 cells. Zn2+ (3 to 300 μM) potentiates ATP(30 μM)-evoked inward current in a concentration dependent manner without changing the maximal response. The mechanism of the facilitation by zinc is different from that by dopamine and high dose of adenosine. Zinc also potentiated ATP(30 μM)-evoked dopamine release in the same manner as that of the currents. The ATP-evoked release was reduced by zinc and shifted the concentration curve of ATP-evoked release to the left without affecting the maximal response. It is suggested that the facilitation is dependent on the calcium influx through ATP-evoked cation channels since it was dependent on extracellular calcium, was blocked by an ATP-receptor blocker suramin, and was parallel to the increase of intracellular free calcium concentration.

636.4  
UPREGULATION OF A1 ADENOSINE RECEPTORS IN CEREBELLAR GRANULE CELLS IN RESPONSE TO CHRONIC COFFEE EXPOSURE. R. D. Hettiger-Smith, M. Leid and T. F. Murray. College of Pharmacy, Oregon State University, Corvallis, OR 97331.

Adenosine is a ubiquitous neurotransmitter in the central nervous system at A1, A2a, and A2b adenosine receptors. Caffeine and related methylxanthines act as non-specific antagonists at A1 and A2a adenosine receptors. Chronic treatment of these G-protein coupled receptors has led to tolerance to the stimulatory effects of caffeine which may, in part, be due to A1 adenosine receptor upregulation. The effect of chronic caffeine exposure in cerebellar granule cell cultures was examined.

Cerebellum was obtained from eight day old Sprague Dawley rat pups and primary cultures of the granule cell neurons were established. Binding in membranes derived from granule cell neurons was examined using the A1 adenosine receptor antagonist [3H]8-cyclopentyl-1,3-dipropylxanthine (DPCCPX). Non-specific binding was determined in the presence of 100 μM 8-cylopentyladenosine (CPA). Receptor expression was analyzed at days 7,9,11 and 13 post culture. Maximum [3H]DPCCPX binding was present at day 13. In order to determine the caffeine concentration needed to obtain maximal upregulation, cells were incubated with final concentrations of 10-1000 μM caffeine and [3H]DPCCPX binding was analyzed. Maximal upregulation of [3H]DPCCPX binding was obtained with 100 μM caffeine. Time course studies revealed that incubation with 100 μM caffeine produced the greatest upregulation of [3H]DPCCPX binding. Cerebellar granule cells in primary culture appear to afford a useful model system for the study of A1 adenosine receptor regulation.
ACETATE DOES NOT INDUCE ADENOSINE-MEDIATED INHIBITION IN THE DENTATE GYRUS OR HIPPOCAMPAL CA1 REGION. J.M. Brundge and T.V. Dumuid.* Dept. of Pharmacology, University of Colorado Health Sciences Center, and Veterans Admin. Medical Res. Service, Denver, CO.

Acetate, the primary breakdown product of ethanol metabolism and can accumulate in the brain following ethanol consumption. It has been suggested that acetate can be metabolized to adenosine within cells in the CNS and release of this adenosine may cause neuronal inhibition. In order to determine whether extracellular acetate on neurons, we applied exogenous sodium acetate (0.5 to 2 mM) to hippocampal slices from adult rat male rats. The acetate caused not significant effect on the resting membrane potential, input resistance, or EPSP amplitude in CA1 pyramidal neurons and had no effect on extracellular field EPSPS in either the CA1 region or the dentate gyrus. To determine the effects of intracellular acetate, we compared the effects of the adenine receptor agonist, thopinephyl on cells impaled with intracellular microelectrodes containing 2.5 M K* acetate, 1M KC1, or 2M K* -methylsulfate. With all three intracellular filling solutions, thopinephyl produced small but statistically significant increase in input resistance, consistent with the presence of low concentrations of extracellular adenosine. However, the response to thopinephyl with acetate-filled electrodes was not different from the other filling solutions. These results suggest that the presence of acetate, either in the extracellular solution or within the intracellular electrode, does not induce a significant adenosine response in the hippocampus. Supported by NS 29173 and the Veterans Administration Medical Research Service.


Increased chloride conductance of mesencephalic cholinergic neurons plays a key role in the production of electrophysiological (EEG) arousal. We recently reported that mesencephalic cholinergic neurons are under the tonic inhibitory control of endogenous adenosine (Balsalobre et al., 1994). Here we report that this state of a drug. We further explored this finding by using the adenosine receptor antagonist, dipyridamole, or exogenous adenosine (10-4 M). We also reported that this adenosine mediated inhibition was reduced in the presence of the adenosine receptor antagonist, dipyridamole, or exogenous adenosine (10-4 M). We also reported that this adenosine mediated inhibition was reduced in vivo.
636.11
TRH ANALOGUES AND ADENOSINE AGONISTS ELICIT VOMITING IN THE CAT. James L. Looce - Dept. Pharmacol., Wright State Univ., Dayton, OH 45435
During the testing of novel receptor actions which theoretically could decrease or select drug effects, some receptor-selective drugs unexpectedly elicit vomiting. Describing novel emetics has value in (1) offering new tools for research into emetic mechanisms, (2) having potential for new insights into the emetic pathways and (3) identifying possible clinical side effects missed by studies with anesthetized subjects or in species which do not vomit. All drugs were administered SC in a volume of 0.1 ml/kg. Tests lasted 30 min or 15 min after the last vomit, whichever occurred later. The disubstituted TRH analogue MK 771 elicited vomiting with an ED50 of 3.1 nM and the monosubstituted CG 3703 did so with an ED50 of 1.9 μg/kg. The adenosine A2 agonist CYS 1986 elicited vomiting with a shallow dose-response curve. The vomiting elicited by 100 μg/kg was blocked by 300 μg/kg of the A2 antagonist CYP, reduced by 100 μg/kg of the nonselective antagonist PD 115199 and not affected by up to 300 μg/kg of the A2 antagonist CYS 1994.

636.12
HISTAMINE ACTION ON ACUITY DESEURTIED NEURONS OF THE RAT NEOSTRIATUM. M. Morikawa and N. Akahile, Dept. of Bi-Pharmacology, Kyushu Univ. Fac. Med., Fukuoka 812, Japan
The neostriatum is innervated by histamine fibers. Histamine receptors have been detected in this region by the radioautographic study and also involved in pathophysiological conditions such as catalepsy and parkinsonism. However, the functional role of histaminergic receptors in the neostriatum remains unknown. We investigated, therefore, the effects of histamine in neurons acutely dissociated from the neostriatum of 2-week-old Wistar rats, using perforated patch clamp technique. In current-clamp mode 10-5 M histamine slowly depolarized the membrane with increased firing activities, and in voltage-clamp mode it evoked a net inward current accompanied by decreased conductance, at a Vm of -44 mV. This response may be restricted in interneurons. We clarified pharmacologically that this histamine-induced response was mediated by both the H1 and H2 receptors. H1 mediated response with a threshold concentration of 10-6 M and reached the maximal response with 10-5 M. Both H1 and H2 receptor-mediated currents resulted from a decreased K+ conductance, which may increase neuronal excitability. The co-existence of H1 and H2 receptors with a wide concentration range may result in extensive modulation of the functional activities in the neostriatum.

636.13
GENOMIC CLONING AND CHARACTERISATION OF A RECOMBINANT HUMAN HISTAMINE H1-RECEPTOR. W.J.M.L. Leyten, M.D. De Baerle, P. Van Gorpel, K. De Leen, W. Gommers and J.E. Leyten, Department of Biochemical Research, Janssen Research Foundation, Beerse, B2404 Belgium.
A human histamine H1-receptor (H1-R) gene lacking introns was isolated by screening a human genomic library with a bovine H1-R probe (BREC 197: 1601-1608 (1993)). The coding region of the human H1-R was designed and subcloned into the pSVL expression vector (Pharmacia) yielding pSVL-H1-R. Membranes from COS-cells transiently transfected with pSVL-H1-R showed aspan-specific binding of [3H]Pyrilamine with a KD of 1.2 nM and a Bmax of 3400 fmol/mg protein (compared to a KD of 0.30 nM and Bmax of 480 fmol/mg protein for guinea-pig cecum membranes).

636.14
MOLECULAR MODELLING OF THE H2 HISTAMINE RECEPTOR. C. Obin-Ayari, M. Ouelts and G. Lunt. Biochemistry Department, University of Bath, Bath, BA2 7AY, UK.
A 3-D model of the canine H2 receptor (1) was constructed and analysed. This model was defined using primary sequence comparisons and three-dimensional homology building, using bacteriorhodopsin as a template. Experimental data from a variety of sources were used to localise the ligand binding site and to identify residues likely to be responsible for receptor affinity, selectivity and efficacy.

636.15
The histamine H3 receptor subtypes involved in the H3 modulation of electrical field stimulated (EFS) neurone responses in pulmonary artery sympathetic and ileum parasymathetic preparations were characterized to determine whether H3A or H3B receptors are present in these tissues. Using techniques of EFS-evoked (F/0) overflow and tension in PHF-noradrenaline-badged pulmonary artery were sensitive to tetradotoxin (300 nM) and insensitive to histamine. In ileum, the evoked tension maximally antagonized by prazosin (100 nM), H3a- methylhistamine inhibition of evoked response and (F/0) overflow were both dose-dependently antagonized by hiperpamin (50-100 μM). In pulmonary artery, the rank order potency of the H3 agonists thiopiperazinom (P2a = 0.1, P2b = 0.4), non-methylated histamine (P2b = 0.7 ± 0.10), and (P2b = 0.7 ± 0.13) was R-a-methylhistamine (P2d = 7.3 ± 1.0) and R-thiopiperazine (P2c = 7.3 ± 1.0). This suggests a predominant H3B-like receptor population on postganglionic sympathetic nerves. In the ileum H3 receptor assay, the rank order potency of the H3 agonists thiopiperazine, butyramine and thiopiperazinom (P2a = 0.1, P2b = 0.4, P2c = 0.7), respectively, was R-a- methy1histamine (P2d = 7.3 ± 1.0), also suggests a predominantly H3B-like receptor population.

636.16
CLOzapine-SENSITIVE BINDING TO HISTAMINE H3 RECEPTOR IN RAT FRONTAL CORTEX. A. Rodrigues, J. Goldfarb and G. Pell*, Dept Pharmacology, Mount Sinai Medical Center, New York, NY 10029
There is evidence suggesting that histaminergic activity is elevated in brains of schizophrenic patients (Soc. Neurosci. Abstr. 18: 446; Lancet 335: 1351; Biol Psychiat. 30: 349). For example, in patients refractory to conventional neuroleptic drugs, CSP levels of histamine's primary metabolite were higher than in controls and correlated with severity of symptoms. In cortex of patients with chronic schizophrenia, H3 receptors were downregulated, consistent with overstimulation by histamine. H3 autoreceptors regulate synthesis and release of histamine; H3 heteroreceptors influence release of dopamine and 5-HT. Neuroleptics bind to H1 and H2 receptors. We examined their interactions with H3 receptors. The latter, in rat cortical membranes prepared in NaK-phosphate buffer (50 mM, pH 7.4), were labeled with H3a-125I methylhistamine. Saturation binding to specific binding defined with either 1 μM thiopiperazine or H3-neo-methylhistamine yielded a Kd of 0.5 nM and a Bmax of 150-170 fmol/mg protein. In competition studies using 0.8 nM H3-125I methylhistamine (specific binding ~90% of total specific binding) with a Ki (assuming competitive inhibition) in the 3-10 nM range, coincident with the range of plasma levels seen clinically. Clozapine's interactions with H3 receptors may have therapeutic relevance. (NS-28012)
636.17 ISTHAMINERGIC NEURONS MEDIATE RESTRAINT STRESS-INDUCED CHANGES IN ACTIVITY OF SELECTED CENTRAL CATECHOLAMINERGIC AND 5-HYDROXYTRYPTAMINERGIC (SST) NEURAL SYSTEMS was examined in male rats. Dopaminergic (DA), noradrenergic (NE) and SST neuronal activity was estimated by measuring concentrations of neurotransmitters and melatonin in brain regions containing these neurotransmitter neurons. Placement of rats within restraining tubes increased dopamine metabolism in the nucleus accumbens, decreased dopamine metabolism in the intermedio-lateral ovoid of the pituitary, and was without effect on the median eminence and median raphe. This data indicate that restraint stress increases mesolimbic, decreases periventricular-hypophysial, and is without effect on nigrostriatal and tuberoinfundibular DA neuronal activity. Neither depletion of neuronal histamine by α-fluoromethylhistidine (αFMH), blockade of H1 receptors by mepyramine, nor blockade of H3 receptors by zolantidine prevented stress-induced increases in dopamine metabolism in the nucleus accumbens suggesting that HA neurons are not major contributors to stress-induced mesolimbic DA neuronal activity. In contrast, treatment with both αFMH and mepyramine, but not zolantidine, prevented stress-induced decreases in dopamine metabolism in the intermedio-lateral ovoid indicating that HA neurons mediate stress-induced decreases in periventricular-hypophysial DA neuronal activity through an action at H1 receptors. Stress increased norepinephrine and 5-hydroxytryptamine metabolism in the hypothalamus, and 5-hydroxytryptamine metabolism in the nucleus accumbens. Both αFMH and mepyramine antagonized, whereas zolantidine did not prevent these increases suggesting that HA neurons contribute to stress-induced increases in SST and NE neuronal activity through an action at H1 receptors. (supported by NIH grant NS31911 and a Pharmaceutical Manufacturers Association Foundation fellowship)

636.19 MELATONIN MODULATES CHOLINERGIC SYNAPTIC TRANSMISSION IN ENTERIC NEURONS B. Prieto-Gomez*, R. Espina-Lana* and C. Barriga-Lopez. 1Department of Physiology, Faculty of Medicine, UNAM, Mexico, 2Department of Biological Sciences, McMaster University, Ontario, Canada.

Melatonin, a hormone produced and released by the pineal gland, is also synthesized by cells of the gastrointestinal wall, where it might be a local regulator of gastrointestinal function. In this study, we investigated the action of melatonin as a modulator of synaptic transmission in the submucosal plexus of the guinea-pig ileum. Intracellular recordings were made in submucosal neurons to measure the amplitude of nicotinic excitatory postsynaptic potentials (EPSPs). Melatonin (10-6 M) reversibly decreased the amplitude of nicotinic EPSPs in a concentration dependent manner (EC50=332 μM). Maximal effects were observed within 4 min after the arrival of the melatonin-containing solution and they persisted for as long as melatonin was present (up to 15 min). Melatonin actions were not modified by the presence of idazoxan and atropine (1μM) indicating that they are not mediated by endogenous release of ACh or noradrenaline or by direct action of α-adrenergic or muscarinic receptors by melatonin. The superfusion of melatonin (1μM) also blocked the nicotinic depolarizations induced by locally applied ACh. These observations indicate that melatonin might be a local modulator of synaptic transmission in the enteric nervous system and that at least part of its effects are postsynaptic. This work was supported by the MRC, OMIH and CANACYT.

636.20 ADENOSINE INCREASES THE SIGNAL TO NOISE RATIO IN FASCIA DENTATA BY A PRE-SYNAPTIC MECHANISM T.M. Swanson*, Department of Neurobiology and Anatomy, Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195.

Adenosine analogs increase or decrease perforant path stimulation evoked dentate granule cell action potential firing depending on concentration. We have previously shown that 10 nanomolar of the stable A1 adenosine analog cyclopentyladenosine (CPA) increases granule cell action potential firing for a given population PSN amplitude. To explore the mechanism of this novel finding, we performed whole-cell current clamp recordings of rat dentate granule cells were performed in 400 μM thick hippocampal slices. Resting membrane potential (-65±4 mV), input resistance (156±32 MΩ), and action potential threshold (±37±3 mV) were similar. Perforant path stimulation were compared in the presence and absence of 1.0 nanomolar CPA in seven experiments. No significant change in any of the measured variables could be detected during CPA application. In other experiments, we measured glutamate release from perforant path stimulated granule cells using a sensitive HPLC assay with FITC- derivitization (resolution limit 10 picomoles). Average basal glutamate release was 170±50 pmol/ml, rising to 450±50 pmol/ml during one Hz perforant path stimulation. No change in glutamate release was detected during CPA application. We conclude that low concentrations of adenosine analogs increase perforant path / granule cell synaptic efficiency without affecting intrinsic post-synaptic membrane properties or altering glutamate release - the data support a pre-synaptic mechanism for this novel action in accord with the documented ability of adenosine to inhibit polysynaptic but not monosynaptic IPSPs in similar preparations.

NEUROTRANSMITTER INTERACTIONS: SEROTONIN

637.1 EVIDENCE FOR INVOLVEMENT OF MEDIAN, BUT NOT DORSAL, RAPHE NEURONAL 5-HT1A AUTORECEPTORS IN THE MEDIATION OF THE ANTICATELEPTIC EFFECTS OF 8-OH-DPAT IN THE RAT. M.L. Wadherbha* and V. Hillegat, Dept. of Behavioral Pharmacology, Astra AB, S-15185 Södertälje, Sweden.

The systemic administration of the 5-HT1A receptor agonist 8-OH-DPAT has been shown to counteract the cataleptic effects induced by DA D2/D3 receptor blocking agents like raclopride or haloperidol. 8-OH-DPAT also produces this effect after i.c.v, but not intrathecal, administration, suggesting a supraspinal mediation of the effect. The dorsal and median raphe nuclei have both been implicated as the target effects for 8-OH-DPAT, but there are divergent opinions on the relative importance of these nuclei in this regard. The present study was designed to clarify the functional role of median versus dorsal raphe 5-HT neurons in the mediation of the antiscataleptic effect of 8-OH-DPAT. Animals were observed for catalepsy on an inclined (60°) grid at different time intervals. Degree of catalepsy was scored according to a square root transformation of raw data. It was found that 8-OH-DPAT (0.5 or 2.5 μg) locally injected into the median raphe nuclei produced a dose-dependent and statistically significant antagonism of raclopride-(16 mg/kg, sc, -h) induced catalepsy. 8-OH-DPAT (0.5 or 2.5 μg) locally injected into the dorsal raphe nuclei, however, had no effect on raclopride-(16 mg/kg sc) induced catalepsy.

In conclusion: The results provide evidence for a specific involvement of the median raphe in the mediation of the antagonism by 8-OH-DPAT of catalepsy induced by dopamine receptor blocking drugs.

637.2 STRIATAL C-FOS INDUCTION BY CONCOMITANT STIMULATION OF 5-HT1A-5-HT2, D1-5-HT1A AND D1-5-HT2 RECEPTORS. J. Depail*, S. Davey*, D. Richard*, and C. Rouillard*. Lab. of Neurobiology, Dept. of Pharmacology and Physiologie, Laval University, Quebec, Canada GIU 1Z4.

We have previously demonstrated that the release of 5-HT by fenfluramine (FEN) induces Fos-like immunoreactivity (Fos-LI) in the doro-medial part of the striatum (STRI) as well as in other brain areas. The striatum, the site of immediate-early gene response is under the control of both DA and 5-HT. However, we were unable to reproduce the effects of FEN in the STR with the administration of selective 5-HT1A receptor subtypes agonist. The present study was aimed to investigate the possibility of interaction between different 5-HT receptor subtypes or between 5-HT and DA receptor subtypes. To investigate this possibility, seven groups of animals, each consisting of at least 3 rats were used in this study. Agonists were administered i.p and animals were sacrificed 120 min later. Brains were removed, sliced and processed for Fos-LI. Treatments were a) Saline, b) Saline+8-OH-DPAT (2.5 mg/kg), c) FEN+8-OH-DPAT, d) Saline+SKF38393 (2.0 mg/kg), e) SKF38393+Saline, f) Skf38393+8-OH-DPAT and g) 8-OH-DPAT+DOI. Our results show that all agonists combinations induce Fos-LI in the medial part of the ST. The crosstalk among 5-HT receptor subtypes. The combination 8-OH-DPAT+DOI was the most effective, followed by SKF 38393+DOI and SKF38393+8-OH-DPAT combinations. No Fos-LI was found in the lateral part of ST. This study suggests that there is a possible interaction and synergestia between 5-HT receptor subtypes and between DA and 5-HT receptors in the ST. The immediate-early gene expression is restricted to a striatal territory related to associative functions while the striatal territory related to mnemonic functions did not show any Fos-LI. (Supported by NSERC and FRQ).
5-HT Receptors Are Not Involved in the 5-HT-Induced Increase of Dopamine in the Ventral Pallidum and Prefrontal Cortex of Awake Rats. J.N. Iyer* and C.W. Bradberry. Department of Psychiatry, Yale Univ. Sch. of Med. and Veterans Administration Medical Center, Box 116124, 195 Campbell Avenue, West Haven, CT 06511.

Interactions between central DA and 5-HT pathways have been proposed to play a role in the pathophysiology of schizophrenia or in amelioration by antipsychotics and in increasing interest in the prefrontal cortex (PFC) as a site of dysfunction in schizophrenia. We have previously shown that the local application of 1-10 μM 5-HT4/5 through a microdialysis probe facilitates DA release in a dose-dependent manner in the PFC. Because previous work suggested that a 5-HT4 receptor sub-type partially mediates a 5-HT4-induced DA release to the nucleus accumbens, we determined if there is 5-HT4-mediated DA release in the PFC.

Injection of the selective 5-HT4 antagonist DOI through a microdialysis probe at a concentration of 500 μM did not cause any increase in extracellular DA and co-perfusion of the selective 5-HT4 antagonist MDL-7097 (100 nM) with 3 μM 5-HT failed to significantly attenuate the 5-HT4-induced enhancement of extracellular DA.

These results demonstrate the absence of 5-HT4 receptor involvement in 5-HT4-induced DA release in the prefrontal cortex of the rat. Supported by DA 00073, NIH 44860, DA 08227, the West Haven Veterans Administration Center Grant for the study of PTSD, and a NARSAD Young Investigator Award to C.W.B.


The role of different serotonin receptor subtypes (5-HT1A, 5-HT2A, 5-HT3) in the control of striatal dopamine (DA) release exerted by serotonin (5-HT) was studied by using in vivo intracerebral microdialysis. A microdialysis probe (CMA 11/11) was implanted in the striatum of rats anesthetized under a mixture of halothane-N2O-O2 and perfused with an artificial CSF (NaCl 145 mM, KCl 2.7, CaCl₂ 1.2, MgCl₂ 1 mM, pH 7.4) at a constant flow rate of 2 μL/min. Two hours after the onset of perfusion, 15 min fractions were collected over 150 min period of time and analyzed by HPLC coupled with electrochemical detection.

In contrast, the control DA release was stable during the experiment.

Drugs used were locally applied by means of the microdialysis probe. 5-HT, 25 and 5pM 5-HT significantly enhanced DA release in a dose-dependent manner up to 157, 255 and 446% of basal value respectively. The effect induced by 1μM 5-HT was not blocked in the presence of 10μM (±)-pindolol, a 5-HT1A receptor antagonist, 1μM ketanserin or 10μM cisapride, both 5-HT3 antagonists, as well as 1 μM domperidone (SR 38029f), a selective 5-HT3 antagonist, were also ineffective. In contrast, 10 μM domperidone (SR 38029f), a selective 5-HT3 antagonist, significantly antagonized the 5-HT-induced DA release.

Moreover, 100 μM BNN 4046, a selective 5-HT3 antagonist, enhanced DA release (+85%) and this effect was reduced by 100 μM D-ala-D-Lys-Enkephalin (40μM).

These results demonstrate that 5-HT-induced enhancement of extracellular DA concentration is mediated by a serotonergic receptor on striatal DA release and that 5-HT3R, but not 5-HT1A, 5-HT2A or 5-HT4 receptor subtypes are implicated in this effect.

5-HT4 Receptors Modulate Histamine Release in the Rat Hypothalamus Measured by In Vivo Micropipette Microdialysis. P.S. Laitinen*, J.T. Laitinen and T. Kuitunen. Department of Pharmacology, University of Turku, 20520 Turku, Finland.

Histamine (HA) acts as a neurotransmitter/modulator in the mammalian brain. HA is thought to modulate circadian functions, including food intake, arousal, body temperature and hormone secretion. In the anterior hypothalamus (AHA), EA is present in the suprachiasmatic nucleus (SCN). A major non-photic input to the SCN is the serotonergic projection from the medial ramus nuclei. Hypothalamic serotonergic neurons (5-HT) also influence the regulation of circadian rhythms, including feeding behavior. We used in vivo microdialysis to monitor the changes in HA and 5-HT in the AHA of male Wistar rats implanted with a microdialysis probe in the AHA (aimed at the SCN). The probe was perfused with mSNP (3 μL/min) and samples were collected every 10 min. The diurnal concentrations of HA was monitored by HPLC with fluorescence detection and that of 5-HT by HPLC-EC.

Local perfusion with 5-HT (10 or 100 μM) increased DA release significantly and dose-dependently. Methysergide (10 μg/ip.), a 5-HT/2 receptor blocker, suppressed basal DA release by 30%. Desfenfluramine, a 5-HT releaser and receptor inhibitor, via the 5-HT (15 μM) increased DA release up to 160 %. With the same dose of desfenfluramine, 5-HT release increased 40-fold in the same brain areas. These data suggest that endogenous serotonin modulates histamine release in the AHA and histaminergic neurons participate in the concomitant effects of desfenfluramine.
637.9 SEROTONIN ENHANCES DOPAMINE-INDUCED INHIBITION OF VENTRAL SEGMENTAL AREA (VTA) NEURONS RECORDED IN VITRO. S.L. Brodie and E.B. Burnet. Dept. Physiology and Biophysics and Program in Emergency Medicine, University of Illinois at Chicago, Chicago, IL 60612.

Dopaminergic neurons of the ventral terminal area (VTA) have been implicated in a number of pathological processes, including drug abuse and schizophrenia. The VTA receives serotonergic innervation from the median and dorsal raphe nuclei, and recent studies have demonstrated that serotonin has both excitatory and inhibitory effects on VTA neurons. We have recently reported that serotonin potentiates ethanol-induced excitation of VTA neurons; our objective in the present study was to determine whether serotonin alters dopamine-induced inhibition of VTA neurons.

Coronal brain slices containing the VTA were prepared from young adult rats. Dopamine (1, 5, 10 pM) inhibited all neurons tested, and was administered in a number of pathogenic processes, including drug abuse and schizophrenia. The VTA receives serotonergic innervation from the median and dorsal raphe nuclei, and recent studies have demonstrated that serotonin has both excitatory and inhibitory effects on VTA neurons. We have recently reported that serotonin potentiates ethanol-induced excitation of VTA neurons; our objective in the present study was to determine whether serotonin alters dopamine-induced inhibition of VTA neurons.

The mean percent inhibition of firing produced by 2 nM dopamine was increased from 38.8% to 48.3% by 50 nM serotonin, and was increased to 53.4% in 10 nM serotonin. Similar effects were seen on the inhibitory potency of 1 and 5 nM dopamine; 5-HT enhancement was statistically significant as determined by ANOVA (P < 0.05). This increase in potency was not explained by an additive effect of serotonin and dopamine, since 1 potention of dopamine effects was seen regardless of whether serotonin alone increased or decreased the firing rate, and 5-HT decrease in firing rate, the effect of dopamine was superadditive with that of serotonin. These data suggest that serotonergic neurotransmission in the VTA may serve to enhance the inhibitory action of dopamine in the VTA. Grant Support: PHS AA-09125; Ulf Helen M. Thomsen Research Fund.

637.10 THE EFFECT OF SEROTONERGIC AGENTS ON THE EXPRESSION OF D1 AGONIST MEDIATED REPETITIVE JAW MOVEMENTS (RJM). Helen Rosengardter and Arnold J. Friedhoff. Department of Psychiatry, Millhauser Labs. NYU School of Medicine, New York, N.Y. 10016 USA.

Selective D1 receptor agonists are capable of inducing repetitive jaw movements (RJM) in rats. SKF 38393 appears to be specific for the D1 receptor, however, at high doses, it is capable of interacting with D1 and 5HT receptors. To explore a possible interaction between serotonergic and dopaminergic systems in the mediation of RJM, we studied the effect of a series of D1 agonists SKF 38393 inducible behavior. It was demonstrated that the mixed 5HT and 5HT receptor agonist, mCPP, and 5HT antagonists, ritanserin and cyproheptadine, are capable of enhancing SKF 38393-induced RJM by 180%, 240% and 217%, respectively, while the 5HT1 agonist, 80H-DPAT, the 5HT1 antagonist, CGS 120066, and the 5HT2 agonist, DDI, had no effect. The results of this study demonstrate that there is an interaction between DA and 5HT systems in the expression of RJM. This study was supported by NIH Grants 06818 and 35976.


The supertransmucous nucleus (SCN) neurons receive excitonic and intrinsic neural inputs, the majority of which utilizes GABA as a neurotransmitter. They also receive serotonergic input from midbrain raphe neurons, which is known to influence SCN neuronal activity through yet unidentified mechanisms. In the present study, whole-cell voltage-clamp recordings were made from SCN neurons in dissociated cell culture, in order to test possible modulation by 5-HT of GABA-induced current (i). With (2-5) of (1)-T and (2) of (5) and (10) of (11), 5-HT enzymatically inhibited i in a concentration-dependent manner (100 nM to 1 mM), without changing the reversal potential of i, which was almost equal to the Cl equilibrium potential. 8-OH-ADPAT, a 5-HT2 agonist, mimicked the effect of 5-HT at concentrations of 100 nM to 10 mM. 5-HT3 (5-HT3 agonist, 100 nM) also inhibited i, whereas a 5-HT3 agonist DOI (1 mM) had no significant effect. The effect of 100 nM 8-OH-DPAT on i was virtually abolished by co-application of mianserin (5-HT3 antagonist, 100 nM) but not by pindolol (5HT1 antagonist, 100 nM). The effect of 100 nM 5-HT was also suppressed by co-application of ritanserin, but not by pindolol or ketanserin (5-HT2 antagonist, 100 nM). External application of 8-Bromo-cAMP (1 mM) or forskolin (500 mM) suppressed i, suggesting that serotonin decreases SCN neuronal activity through a receptor system other than the GABAergic-cAMP pathway. The results suggest that 5-HT inhibits i in the SCN neurons, which involves the activation of 5-HT receptor and its coupled cAMP-dependent system. This inhibition of GABA_A receptor function may be involved in the regulation of 5-HT of SCN neuronal activity.


Serotonin (5-HT), substance P (SP) and tryptophan-releasing hormone (TRH) occur in ventral medial neurones which project to the intermediolateral cell column (IML) of the thoracic spinal cord (Sasek et al., Neurochemistry, 1976. 10, 951). These fibers are involved in the regulation of sympathetic nervous system. 5-HT-[3H]-HT is released from the intermediate area which includes the IML and the release of the 5-HT is regulated by presynaptic inhibitory 5-HT receptors (Yamaguchi, et al., Brain Res., in press, 1994). We used in vitro superfusion of the microdissected intermediate area of the rat thoracic spinal cord to study the effects of SP and TRH on the release of 5-HT. A serotonin (100 nM, the preferred receptor for SP) selective agonist, GR 73632 (1 nM), significantly increased the basal release of 5-HT. The increased release of 5-HT by GR 73632 was blocked by a selective NK, antagonist, GR 83341 (1 nM). GR 83341 (1 nM) did not change the basal release of 5-HT at itself. SP (1 nM) also increased the basal release of 5-HT. A TRH agonist, NK 771 (1 nM), had no effect on the release of 5-HT. The present study suggests that release of 5-HT the intermediate area could be regulated by SP, but not by TRH. The excitatory effect of SP on the release of 5-HT appears to be mediated by NK receptor. These findings will be helpful in understanding the complex roles of coexisting neurotransmitters in the spinal regulation of the sympathetic nervous system. (Supported by NIH grants DE 007896.)


Many serotonin-containing fibers that terminate on or near motoneurons in the ventral horn of the spinal cord and the hypo- glossal nucleus contain colocalized substance P. A significant proportion of fibers in the same regions appears to contain colocalized serotonin and enkephalin. Rapidly firing serotonin cells appear to corelease both serotonin and neuropeptides. Enkephalin is known to affect these substances interact to affect postsynaptic cells. The present study used electrophysiological techniques to examine how serotonin may interact with these neuropeptides to alter motoneuron excitability in the lumbar spinal cord and in the hypoglossal nucleus. Microiontophoretic application of serotonin or substance P enhanced glutamate-evoked firing of all motoneurons that were tested in the spinal cord and the hypoglossal motor nucleus. Application of serotonin and substance P together had additive effects on motoneuron excitability until a threshold was reached. In contrast, microiontophoretic application of enkephalin inhibited glutamate-evoked firing of the same cells that were facilitated by serotonin. Concurrent application of serotonin and enkephalin diminished the facilitatory effects of serotonin alone. Therefore, it appears that cells which contain colocalized serotonin and enkephalin may either enhance or inhibit motoneuronal excitability depending upon the firing rate of the cell.

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637.15
IS TRYPTAMINE SYNTHETIZED BY SEROTONERGIC NEURONS?
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Tryptamine (T) is present in the Central Nervous System (CNS) in very low concentration. T is synthesized from Tryptophane (Trp) by the aminoacid aromatic decarboxilase (AADC), an enzyme that is present in all eumammalian neurons and also in glial cells. Localization of T in serotonergic neurons is not yet clear. An immunocytochemical technique (ICT) was applied to CNS of adult rat to detect anti-serotonin and primary antibody. To increase the concentration of both endogenous and exogenous TGF, the animals were treated with Trp and pergolide was performed 2 hr prior to fixation. T immunoactivity was restricted to soma and processes of glial and neuron cells in the raphe nucleus (pere reticulate), and pons giganito cellularis nucleus. We discovered, therefore, that T can be synthesized by glial and other cells in the CNS. Distribution of serotonergic cells does not lay on top of tryptamine cells in the CNS, detected by ICT, so not all serotonergic neurons are able to synthesize T. Immunoactivity for both indolesamines was strongly reduced by inhibition of serotonin synthesis with parachlorophenylalanine pre-treatment. We postulate that synthesis of T is not an alternative route for Trp utilization when TGF-hydroxylase (limitant enzyme of serotonin synthesis) is inhibited. (Supported by grants of CONICET and UBA/CT, Argentina).

638.1
DIHYDROXIDINE RELEASES ACETYLCHOLINE AND IMPROVES COGNITIVE PERFORMANCE IN RATS.
Neurochemical and behavioral studies have elucidated extensive interactions between dopaminergic and cholinergic systems in brain areas associated with movement and cognition. The initial goal of these studies was to evaluate the effect of the anti-Parkinson drug dihydriodicine (DHX), a full D2 agonist, on brain acetylcholine (ACH) release using in vivo microdialysis techniques. Moderate doses (3 & 10 mg/kg) of DHX produced an approximate 50% increase in striatal ACh release that was blocked by the D2 antagonist SCH23390 (0.3 mg/kg). A higher dose (17.5 mg/kg) was less effective in raising striatal ACh release probably due to DHX neurotoxicity. In frontal cortex, DHX (10 mg/kg) evoked a more robust increase (200%) in ACh release that was blocked by SCH23390 (0.3 mg/kg). Since elevations in brain ACh are associated with cognitive improvement, the effectiveness of DHX in a passive avoidance model of learning and memory was evaluated. These studies revealed a significant improvement in performance by 0.3 mg/kg DHX in scopolamine-amnesic rats. These results provide support for the hypothesis that DHX improves cognitive performance as a consequence of ACh release in relevant brain regions. Further, D2 agonists may have novel therapeutic potential in the treatment of dementia.

638.2
RELEASE OF [3H]ACETHOLLINE FROM SLICES OF RAT STRIATUM AND CEREBRAL CORTEX BY 5-HT, RECEPTOR ANTAGONISTS. J. Del Rio*, B. Laesther and M.J. Ramirez Dept. of Pharmacology, Schools of Medicine and Pharmacy, University of Navarra, 31080-Pamplona, Spain.
Controversial results have been reported on the ability of 5-HT receptor ligands to modulate acetylcholine (ACH) release from rat entorhinal cortex and also from other brain regions. We have studied the effect of 5-HT1 receptor stimulation and blockade on basal and K+ evoked [3H]ACH release from superfused slices of rat striatum and cerebral cortex. K+ evoked release consisted of two stimulations (S1 and S2, 30 min apart) with KCI (20 mM, 6 min) with drugs added 5 min before S1 and calculation of changes in S2/S1. The 5-HT1 antagonist ondansetrone (0.01-10 µM) increased in a concentration-dependent manner both basal and K+ evoked [3H]ACH release from striatal slices, the latter effect being more marked. A lower though significant [3H]ACH release by ondansetrone was also found in superfused cortical slices. However, basal rather than K+ evoked [3H]ACH release was influenced by this 5-HT1 antagonist in the cerebral cortex. Similar effects were observed with higher concentrations of gantrisent in both brain regions. The 5-HT1 agonist 2-Me-5-HT (1 µM) did not modify by itself [3H]ACH release and a moderate decrease was only observed in the presence of the 5-HT1 antagonist ritanserin. Interestingly, 2-Me-5-HT blocked the increase in both basal and K+ evoked [3H]ACH release induced by ondansetrone. The results suggest that 5-HT1 receptor antagonists may influence acetylcholine release from specific brain regions.

638.3
PRETREATMENT WITH THE NORAADRENERGIC NEUROTOXIN DSP-4 IN VIVO REDUCES ACETYLCHOLINE (ACH) RELEASE IN THE RAT PREFRONTAL CORTEX IN VITRO. Stéphane Teillez, Francis Colpaert and Marc Marien*, Centre de Recherche Pierre Fabre, 17 avenue Jean Moulin, Castres 81100, France.
A deficiency in the locus coeruleus noradrenergic system (LC-NA) has been proposed to be an important contributory factor in the pathogenesis and progression of central neurodegenerative disorders including Alzheimer's disease (Colpaert 1994, in Noradrenaline Mechanisms in Parkinson's Disease, CRC Press, Boca Raton, pp. 225-254). To examine the influence of the LC-NA on cortical ACh release, male SD rats were injected with DSP-4 (40 mg/kg, i.p.) preceded 30 min by an injection of citoplatin (10 mg/kg). Three days later, slices of the prefrontal cortex (PFC) were prepared, incubated with [3H]ACh, superfused and stimulated by exposure to increasing concentrations of K+. The DSP-4 treatment reduced endogenous noradrenaline levels in cortical tissue by 71-94%, in slices of PFC from DSP-4 treated rats, the [3H] overflows which were stimulated by 20, 35 and 45 mM K+ were only 32, 25 and 22%, respectively, compared to slices prepared from vehicle treated animals. These results indicate that disruption of the LC-NA reduces a facilitatory input to the cortical cholinergic system, and that injury to or loss of noradrenergic neurons might contribute to the cortical cholinergic dysfunction in Alzheimer's disease.

638.4
CHRONIC EFFECTS OF CAFFEINE, NICOTINE AND ETHANOL ON CENTRAL ADENOSINE AND CHOLINERGIC RECEPTORS AND CALCIUM CHANNELS: BEHAVIORAL CORRELATES IN MICE. Dan Shi, O. Nikolaevic, B. Justo, K. Jacobson and I. M. Daly, Laboratory of Bioorganic Chemistry, NIDDK, NIH, Bethesda, MD 20892.
Chronic caffeine increases density of central A1-adenosine (cortex, striatum), nicotinic, muscarinic, serotonin and GABAergic receptors. Central A2A-Adenosine, δ-adrenergic, dopaminergic and NMDA-receptors are unchanged and the densities of β-adrenergic receptors are decreased. Theophylline and theobromine also increase density of A1-receptors. No changes are observed to adenosine receptors or muscarinic receptors. Chronic ethanol increases density of A1-receptors. Both caffeine and ethanol, but not nicotine, increase muscarinic receptors. Behavioral sensitivity (open field locomotor) to adenosine agonists increases after caffeine. Behavioral sensitivity to nicotine decreases after chronic administration. The sensitivity to the muscarinic antagonist scopolamine is enhanced after chronic administration of caffeine. Adenosine receptor agonists appear interrelated behaviorally as are 1-type calcium channels.

Ventral pallidal regions of the basal forebrain (VF) contain cholinergic neurons that project to the prefrontal cortex (pFC), frontal cortex (FC) and amygdala (AMG) and are involved in cognition. Cholinoceptive neurons of the medullobulbar pontine nucleus (PPN) are directly innervated by non-cholinergic neurons of the VF that mediate motoric function. Our previous work demonstrated that intra-VF injections of dopamine (DA) and its receptor-subtype selective agonists alter motor function, but do not effect working memory (i.e., choice accuracy on the radial arm maze). It is not known whether cholinergic neurons within the VF, or cholinergic neurons innervated upon by VF efferents, are affected by these treatments.

In the present study, changes in hemicholinium (HC-3)-binding, a marker for high affinity choline uptake, were measured in pFC, FC, AMG and PPN of tissue homogenates as an indicator of cholinergic neuronal activity. Intra-VF microinjection of DA failed to alter HC-3 binding, but DA(+50, 5µg per side) increased binding by 25% in the PPN. In contrast, 5,6-DHT-induced lesions of the ascending DA projection decreased HC-3 binding in the pFC and AMG 36% and 24%, respectively. These data indicate that impact of VF cholinergic systems influenced by the VF are differentially sensitive to 1) DA receptor stimulation within the VF, and 2) the compensatory mechanisms evoked upon removal of endogenous DA. Work supported by MH413180 to TCN.


After medial septum lesions (MSL), peripheral sympathetic adrenergic fibers, originating from ventral ventrolateral tegmentum, enter the hippocampus (hippocampal sympathetic interneuron,HSI). We have previously reported that HSI is detrimental to learning in rats and that treatment with phentolamine (PHENT), a-adrenergic antagonist, ameliorated this deficit. However, biochemical studies of HSI have revealed changes in the hippocampal cholinergic system. To determine whether PHENT treatment might mediate its effects through muscarinic cholinergic (mAChR) receptors, we assessed the efficacy of PHENT treatment on hippocampal mAChR. Animals underwent 1 of 4 surgeries: control (sham MSL + shm ganglionectomy (Gx)); HSI (MSL + shm Gx); HSI (+- MSL + Gx); sham (MSL + Gx). Each surgery group was divided equally between vehicle (20 mg/kg, IP) or vehicle daily for 5 weeks. After completion of treatment, the hippocampus was isolated from the dorsal and ventral regions and membrane-binding of [3H]QNIX, a non-selective mAChR antagonist, was assessed. PHENT treatment was found to reduce the binding affinity of mAChR in HSI(+)-animals in both hippocampal regions (Kd: 0.65 ± 0.38, 1.08±0.25 μM) in comparison to controls (Kd: 0.48±0.06, 0.54±0.05) and 2) increase the number of mAChR in dorsal hippocampus of HSI(-) animals (Bmax: 1111±416 fmol/mg) in comparison to control animals (Bmax: 409±50.7) These results suggest that PHENT treatment may mediate its effect through the mAChR by altering the release of acetylcholine from intrinsic cholinergic neurons and indicate that balance between noradrenergic and cholinergic system may be important for mediating the behavioral effects of HSI.

538.8 CALRETININ IMMUNOREACTIVITY IN THE ADULT RAT SEPTO-HIPPOCAMPAL BARRIER PATHWAY. L.A. Wilson and M.D. Kaye*, Department of Anatomy and Cell Biology, Queen’s University, King’s College, Kingston, Ontario, Canada, K7L 5N6.

The medial habenula (MBH) consists of a densely packed population of neurons, the majority of which stain immunohistochemically for choline acetyltransferase (ChAT). The cholinergic MBH is innervated by axons that originate from pontine cholinergic nuclei (PCh), and which project via the stria medullaris (Sm). To date, one of the few biochemical features to be identified within this nucleus is it’s calcium–binding protein. The purpose of this investigation is to determine the normal patterns of connectivity of calretinin-positive fibers in the MBH, as a prelude to our examination of the role of calretinin in the adult habenula. A denseplexus of calretinin-immunoreactive axons was evident throughout the rostro-caudal extent of the normal MBH. Following a unilateral lesion of the Sm, calretinin immunostaining of fibers was completely abolished in the ipsilateral MBH, a small population of calretinin-positive neurons remained in the MBH, and no calretinin staining was found in the intact contralateral MBH. As an extension to these findings, these immunoreactive terminals formed asymmetric synapses with dendritic processes of MBH neurons. Double immunohistochemical staining was used to simultaneously demonstrate calretinin-positive fibers among CHAT-positive neurons within the ventral two-thirds of the MBH. The data will be presented concerning the ultrastructural organization of CHAT and calretinin immunoreactivities within the normal adult rat MBH and within implants of fetal rat habenula grafted into the mature rat brain (Supported by funds from the Faculty of Medicine, Queen’s University).

538.9 INTERACTION OF SOMATOSTATIN AND CARBACHOL ON HIPPOCAMPAL MAB CR RECEPTORS. P. Cagliari, M. Schilling, M. S. Bagg and L. Sigl. Department of Neuropharmacology, The Scripps Research Institute, La Jolla CA 92037.

The M-current (I(M)) is a non-inactivating potassium current that persists at slightly depolarized potentials. In CA1 hippocampal pyramidal neurons, somatostatin (SS) increases I(M) whereas carbachol (CCh) decreases it. To further study the ionic mechanisms of I(M), we investigated the interaction of these two substances on I(M) using intracellular voltage-clamp recordings in the rat hippocampal slice preparation. We recorded from 26 neurons with a mean resting membrane potential of -48 ± 8 mV. Suppression of CCh at 1 µM or 0.25% of control, an effect greater than previously reported. We then were able to reverse the CCh inhibition of I(M) by adding SS at 1 µM together with CCh: SS had no effect and I(M) remained inhibited. Reversing the order of agonist application gave different results. When SS 1 µM was added first, it was increased by 55-75%, as previously reported. Then adding CCh 1 µM to the SS-summated I(M) raised its amplitude back to 85-95% of control. As SS did not decrease I(M) below control in the presence of CCh alone, SS thus provides a protective effect. However, when we applied a high concentration (30 µM) of CCh in the presence of SS, the I(M) amplitude decreased greatly (15-25% of control), an effect identical to the effect of CCh on I(M) alone. Thus, in rat hippocampal CCh prevailing over SS for the control of I(M). However, SS can protect some of the M-channel pool against low (1 µM) but not high (30 µM) concentrations of CCh. Concentration and sequence of superfusion determines the end-effect, the interaction between these two agonists could take place at the second messenger level. Supported by NIMH (MH 44346) and NIAAA (AA 07456).

538.10 IMIDAZAZEN AND ABDOSULIN, TWO ANXIOLYTIC SELECTIVE GABA RECEPTOR MODULATORS REDUCE STRESS-INDUCED RELEASES OF ACETYLCHOLINE AND DOPAMINE IN THE RAT BRAIN. L. Depoix, C. Motto, A. Importer, M. Serra and S. Biggio*. Departments of Experimental Biomedical and Neuroscience, University of Cagliari, 09122 Cagliari, Italy.

The effect of the partial agonist imidazolin (IM) and the selective agonist abeculine (AB) was studied on the basal and stress-stimulated release of acetylcholine and dopamine in the rat hippocampus and cortical cortex using the microdialysis technique on freely moving rats. The effect of these drugs was compared with that of diazepam (DZ) and alprazolam (AZ), the two benzodiazepines full agonists. AB (0.05 - 1 mg/kg p.o.), IM (0.05 - 1 mg/kg p.o.), DZ 0.25 - 10 mg/kg p.o. and AZ (1 - 10 mg/kg p.o.) produced a dose-dependent decrease in the kynurenine, a calcium–binding protein. On the other hand only DZ and AZ significantly decreased dopamine release in the c. cortex. These effects were augmented by flumazenil. Fast onset (0.2 mg/kg/1000 mg/kg) produced a slowly marketed and marked 75-85% inhibition of acetylcholine release. The effects of AB were maximal at 30 min and remained at basal values in about 80 min. Fast shock enhanced also dopamine release in the c. cortex. The maximal effect (100%) was obtained at 30 min and in 30-40 min dopamine release returned to control values. The preexposure administration of AB or IM in a dose that induced the baseline release of acetylcholine and dopamine prevented the effect of stress. An effect mimicked by much higher doses of DZ and AZ. The differential efficacy of IM and AB on acetylcholine and dopamine release is consistent with the existence of multiple GABA, receptor subtypes with different sensitivity to these drugs. Supported by CNR subproject: SP4 stress CNR 93.0592.PF41.

Behavioral and biochemical studies suggest that a negative interaction exists between adenosine (ADO) A2 and dopamine (DA) D2 receptors in the brain and that this interaction underlies the opposing effects of ADO and DA receptors in vivo. ADO agonists are localized to GABAergic striato-pallidal neurons, and we have shown that activation of these receptors increases electrically-evoked underevoked GABA release in microdialysis from globus pallidus (GP). We examined the functional significance of A2A and D2 receptor subtypes in modulating electrically-evoked GABA release. We have observed that D2 receptor stimulation reduces the extent of co-release of GABA and glutamate from these cells, while A2A receptor agonists stimulate GABA release in microdialysis from caudate nucleus (CN). We found that the A2A receptor antagonist CGP 42226 (1 mg/ml) reduces the extent of GABA release in microdialysis from the caudate nucleus, and increases the extent of glutamate release from the same area. We also found that A2A and D2 receptor subtypes have opposing actions on pallidal GABA release. Consistent with previous findings, GABA release was increased by 35-40% by the selective A2A receptor agonist CGS 21680 (10 nM). The selective D2 receptor agonist N-0437 (1-100 nM) resulted in a concentration-dependent decrease in evoked GABA release in microdialysis from GP as well as in striatal slices (containing GP). GABA release was approximately 30% in both tissue preparations. However, in the presence of 10 nM CGS 21680, N-0437 (1-100 nM) had no effect on evoked GABA release. These results demonstrate that agonist stimulation of ADO and DA receptor subtypes has opposing actions on pallidal GABA release and that the stimulation of A2A receptors abolishes the effects of D2 receptor stimulation. It is suggested that this functional interaction between A2A and D2 receptor subtypes represents an important mechanism by which GABAergic striato-pallidal output is differentially modulated by ADO and DA. (Supported by NS 26831)

Zn2+ MODULATES GABAERGIC TRANSMISSION IN ORGANOTYPIC SLICE CULTURES OF RAT HIPPOCAMPUS. J.N. Record* and A.T. McLennan. Neurological Surgery, RI-20, University of Washington, Seattle, WA 98115.

Intracellular recordings of CA3 pyramidal cells (PCs) were used to study the actions of Zn2+ in slice cultures of hippocampus. Zn2+ (100-300 MuM) caused spontaneous and stimulus-evoked giant depolarizing potentials (GDPs) consisting of a burst of action potentials followed by depolarization (see Xia and Smart 1993).J.Physiol. 460-503). Paired recordings of neighboring PCs showed simultaneous GDPs, suggesting synchronous synaptic drive. Blockade of synaptic transmission using either 1 MuM TTX or 100-300 MuM cadmium prevented the appearance of GDPs. Glutamate and GABA receptor antagonists were used to examine the contributions of these neurotransmitters to GDP initiation and topography. The glutamate receptor antagonists APV, CNQX, and the GABA(A) antagonist CGP-33348, resulted in the appearance of spontaneous and evoked depolarizations. The depolarizing potentials appeared to be GABA-evoked, since they were reversible near resting membrane potential. Application of Zn2+ in the presence of APV, CNQX, and the GABA(B) antagonist CGP-33348, resulted in the appearance of spontaneous and evoked hyperpolarizations. These large hyperpolarizations were blocked by CGP-33348, suggesting they are GABA(B) receptor-mediated IPSPs. These data suggest that Zn2+ enhances GABA, and GABA-mediated neurotransmission in organotypic cultures of hippocampus. The appearance of GDPs appears to result primarily from the enhancement of GABA(A) transmission. (Supported by NIH-NSS2630 and NIH-T32NS-0144-15.)

COLOCALIZATION OF GLUTAMATE DECARBOXYLASE (GAD), SEROTONIN (5HT) AND TYROSINE HYDROXYLASE (TH) IN RAT MEDIAL PREFRONTAL CORTEX (mPFC). J.D. Taylor* and F.M. Berens. Department of Psychology and Program in Neuroscience, Harvard Medical School and Lab for Structural Neuroscience, McLean Hospital, Belmont, MA 02178.

Recent evidence has suggested that GABAergic cell bodies in rat prefrontal cortex receive a convergent modulation of monoaminergic inputs (Gellman and Aghajanian, 1993). To obtain corroborative microscopic support for this idea, a fluorescent immunocytochemical technique has been developed for the colocalization of GAD, TH and 5HT in single sections of rat mPFC. The primary antisera included a polyclonal antibody raised in rabbit against GAD, a monoclonal antibody raised in mouse against TH and a polyclonal antibody raised in goat against 5HT. Secondary antibodies were raised in donkey against rabbit (FITC), mouse against goat (TRITC) and goat against rabbit (AMCA). A digital confocal microscopic system equipped with appropriate filters was used to visualize the three fluorescent emissions. For a given cell of interest, serial 2 a/s plane slices were corrected for background fluorescent using a deconvolution subroutine followed by co-registration of the three images. While TH and 5HT varicosities were found in apposition with GAD immunoreactive (IR) cell bodies, ghosts of pyramidal neurons also showed a "divergence" of TH-, 5HT- and GAD-IR varicosities/boutons. Interactions of TH-, 5HT- and GAD-IR elements with one another were also commonly observed pre-synaptic to pyramidal cell bodies. These findings are consistent with the idea that monoaminergic afferents converge on GABAergic and pyramidal cell bodies. Moreover, pre-synaptic interactions of monoaminergic varicosities with GABAergic elements may play a significant role in the inhibitory modulation of pyramidal cell output from mPFC. Supported by MH42261, MH00423, MH11514 and the Stanley Foundation.


Recent evidence suggests that GABA and glycine are released by the same local circuit neurons in the spinal cord (Todt and Sullivan, J. Comp. Neurol. 296:496-505, 1990). Among the most important questions is whether this is a general phenomenon. However, few data are available in this regard, and most laboratories working with this system have focused on single neurotransmitters. We used a combination of immunocytochemical and electrophysiological methods to study the colocalization of GABA and glycine in the cuneate nucleus, 29% stain for GABA, 29% stain for glycine, and 42% stain for both.

Electron microscopy showed that GABA colocalizes with glycine at both ascorndendritic and axosomatic synapses, supporting a role for these neurotransmitters in pre-synaptic as well as post-synaptic interactions. Most GABAergic terminals were also glycergic, but some terminals were clearly not, as was GABA and not glycine, and vice versa. We are now studying quantitative differences in the enrichment of GABA and glycine in various classes of terminals in the cuneate nucleus.
638.17

We previously reported the localization of immunoreactivity to the amino acid transmitters GABA, glutamate (GLU), and glycine (GLY) in the chicken retina. In this report we assess the colocalization of these transmitters in the chicken retina inner nuclear layer.

Posthatch chicks were deeply anesthetized (halothane), decapitated, and their retinas fixed by immersion in mixed aldehydes. Small strips of central retina were embedded in epon and sectioned transversely at 1.5 μm. Adjacent serial sections were mounted on different slides to compare sections through the same cell immunoreactive to primary antibodies, (Chemicon) GLU, GABA, or GLY, visualized using an ABC kit (Vector).

Horizontal sections were used. Most GLU+ cells were also GABA+. A few GABA+ cells were GLY+. Bipolar cells were virtually all GABA+ and those which were both GLU+ and GLY+ were often adjacent to horizontal cells. Amacrine cells stained heterogeneously. GLU+ cells were most numerous followed by GLY+. Fewer of the GLU+ cells were GABA+, but most GABA+ cells were also GLU+ or GLY+, suggesting that at least some of these cells may contain all 3 transmitters.

The colocalization of GABA and GLY in some horizontal cells, the colocalization of GLU in GLY+ bipolar cells, and the probable colocalization of all 3 among some amacrine cells raises the question of possible functional and morphological differences among these cells.

(Partial support for W.J.C. Mich. Eye Bank & Transplant Center)

639.1
THE EFFECTS OF SUBSTANCE P (SP) AND SEROTONIN (5-HT) ON DOPAMINE (DA) RELEASE IN THE STRIATUM: MICRODIALYSIS STUDIES IN WO. Camille S. Suchowski* and Matthew P. Galloway, CCN Program, Dept. of Psych & Behav Neurosciences, Wayne State Univ Sch of Med, Detroit MI 48202.

Several studies have demonstrated that 5-HT facilitates DA release in vivo, however the 5-HT receptor subtype and the synaptic organization remain to be determined. Using microdialysis, we analyzed the potential involvement of 5-HT1B receptors and the effects of the neurokinin, SP and senktide (a NK3 agonist) on extraneuronal striatal DA levels. Local perfusion with 10μM 5-HT significantly increased DA levels approximately 7-fold above baseline (655±71%, n=6). After perfusion with 100μM cyanoacrylic (CYP), a 5-HT1B antagonist, baseline DA levels increased 91%. In the presence of CYP, 10μM 5-HT no longer had a significant effect on DA levels (n=5). Persusion of 100μM SP did not significantly alter extracellular striatal DA levels (n=7) nor did local perfusion of 100μM senktide (n=6). To determine whether SP interacted with the effect of 5-HT, tissues were perfused with 100μM SP or senktide followed by a 10μM pulse of 5-HT. SP or senktide pretreatment did not alter 5-HT’s effect on extracellular DA levels (n=11). Preliminary microdialysis experiments measuring extracellular DOPA after perfusion diffusenilthelydopa, a decarboxylase inhibitor, indicated that 100μM senktide increased DOPA levels by 98% (n=1). In summary, 5-HT induced DA release was blocked by CYP. Persusion of SP and senktide did not significantly alter DA release or alter the 5-HT facilitation of DA release. Supported by NIDA 04120 and the Joe Young Sr. Research Fund.

639.3

In the present study we have investigated the effects of various classes of excitatory amino acid agonists on the release of dopamine in the medial prefrontal cortex of conscious rats. Bilateral microdialysis probes were used to assess extracellular levels of dopamine and to infuse excitatory amino acid agonists locally. Infusion of 20 μM kainate resulted in behavioral stimulation (e.g., sniffing) and a sustained, robust, nearly 7-fold increase of the extracellular levels of dopamine. Local application of 20 μM AMPA had a less profound effect, causing an increase of 2- to 3-fold in extracellular levels of dopamine. Higher concentrations of AMPA (100 μM) increased dopamine levels by nearly 10-fold. However, this increase was associated with convulsions in most animals tested. Local infusion of 20 μM NMDA did not affect extracellular dopamine levels. A much higher concentration of NMDA (200 μM) led to a 3- to 5-fold increase in dopamine levels. In contrast to AMPA or kainate, higher concentrations of NMDA did not cause convulsions. These results indicate that excitatory amino acid agonists facilitate the release of dopamine in the prefrontal cortex in vivo, and that non-NMDA receptor agonists are more potent than NMDA receptor agonists in so doing. This finding is in agreement with our previous 5-HTA receptor antagonists can attenuate the release of dopamine in the prefrontal cortex during stress. Supported in part by MH84804 and MH48866 and the Scottish Rite Foundation.

639.4
DOPAMINE RELEASE IN ANTERIOR MEDIAL STRIATUM IS CONTROLLED BY THE PREFRONTAL CORTEX: EVIDENCE FOR TONIC AND PHASIC MODULATION. M. Karremann* and B. Moghadam, Dept. of Psychiatry, Yale University School of Medicine and West Haven VA Med. Ctr. 116A/2, West Haven, CT 06816.

In the present study, we have investigated whether the prefrontal cortex exerts a modulatory effect on dopamine release in the anterior medial striatum of conscious rats. Two concentric microdialysis probes were used in each animal: one placed in the medial prefrontal cortex, used primarily for drug infusion, and the other placed in the contralateral anteromedial striatum, used to measure extracellular levels of dopamine. Infusion of TTX (5 μM) into the prefrontal cortex caused a decrease in extracellular dopamine levels in the anterior medial striatum, indicating that neuronal events in the prefrontal cortex exert a tonic excitatory effect on striatal dopamine release. Infusion of bicuculline (100 μM) into the prefrontal cortex resulted in an increase in the release of dopamine in the striatum, while application of amphetamine (50 μM) in the prefrontal cortex attenuated dopamine release in the striatum. These data suggest that GABAergic, as well as monoaminergic, systems in the prefrontal cortex control striatal release of dopamine. Further pharmacological characterization of tonic and phasic cortical mechanisms that modulate the release of dopamine in the striatal complex are currently underway.

Supported in part by PHS grants, MH84804 and DA08227
639.5
THE 5-HT2ANTAGONIST AMPEROZIDE POTENTIATES AMPHETAMINE-STIMULATED DOPAMINE RELEASE IN THE RAT PREFRONTAL CORTEX. E.A. Felkai and H.Y. Meltzer. Department of Psychiatry, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

The pharmacological profile of the putative atypical antipsychotic drug amperozide includes 5-HT2 antagonism coupled with the ability to block dopamine (DA) uptake. Both of these properties may be the basis for the reported ability of amperozide to attenuate amphetamine (AMPH)-stimulated DA release in the dorsal and ventral striatum. Since previous research has not demonstrated a significant difference between the mesostriatal and mesocortical pathways, it is important to compare the regulation of DA release between these systems. The present study thus examined the effects of amperozide administration on AMH-stimulated DA release in the mesoaccumbens and mesocortical pathways.

Male rats were implanted with chronic indwelling guide cannulas above the medial PFC. 2-3 days later, microdialysis probes were lowered through the PFC into the rostral 5-HT2a receptor rich area. Following collection of baseline samples, amperozide (1.0, 5.0 or 10.0 mg/kg i.p.) or saline were administered 30 min before vehicle (0.3 mg/kg i.p.). AMPH increased basal extracellular DA levels to 0.88 pg/20 uL to 4.35 pg/20 uL 30 min following injection. Amperozide administration did not attenuate this increase. Rats that received amperozide potentiated AMPH-stimulated DA release in a dose-dependent manner. Relative to AMPH alone, administration of 1.0, 5.0 and 10.0 mg/kg amperozide produced additional increases in DA efflux of 1.06, 2.44 and 4.05 pg/20 uL, respectively. These results may be due to actions of amperozide on the cortical 5-HT2 receptors coupled with effects on AMPH on the DA transporter. Furthermore, these data add to previous work demonstrating differences between the mesostriatal and mesocortical systems in the regulation of DA release.

639.7

(+)-MDMA (3,4-methylenedioxymethamphetamine, "Ecstasy"), an amphetamine related drug of abuse, is a potent releaser of serotonin (5-HT) and causes toxicity to 5-HT neurons after repeated exposure. (+)-MDMA also releases dopamine (DA), although with less potency. Since we have shown previously that the intrastriatal application of (+)-MDMA facilitates DA release via a mechanism that is not observed with either (+)-MDMA or 5-HT, we investigated the role of endogenous 5-HT in (+)-MDMA-induced DA release in vivo.

Using the 5-HT1B/1D antagonist 8-OH-DPAT (0.3 mg/kg s.c.) or (+)-MDMA (1.0 mg/kg i.p.), extracellular DA levels were monitored in the substantia nigra pars reticulata (SNpr), the ventral tegmental area (VTA), the dorsal raphe nucleus (DRN), and the nucleus accumbens (NAcc) in anesthetized rats. (+)-MDMA increased DA efflux in all rostrolateral forebrain regions. (+)-MDMA increased DA efflux bilaterally in the SNpr and DRN. These increases are synergistic with the DA efflux induced by (+)-MDMA administered in the VTA and DRN. These data suggest that the (+)-MDMA-induced release of DA in the SNpr and DRN is not mediated by 5-HT1B/1D receptors.

639.9
EXTRACELLULAR DOPAMINE IN NUCLEUS ACCUMBENS SHELL IS ALTERED BY 6-OHDA LESIONS OF PREFRONTAL CORTEX. D. King*, M.J. Zigmund, and J.M. Finn. Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

We previously reported that neither basal nor evoked extracellular dopamine (DA) is measured in the prefrontal cortex (mPFC) DA depletion (King et al., Soc. Neurosci. Abst. Fr19:29). In the present study, the impact of such lesions on extracellular DA in the nucleus accumbens shell, and core, were examined using in vivo microdialysis in 6-OHDA (1 µg/g saline) and saline injected rats. 30 min after injection of 6-OHDA, mPFC were performed 30 min after treatment with desipramine (25 mg/kg). Two weeks later, a microdialysis probe was inserted into the 6-OHDA-treated hemisphere and placed into shell or core. Approximately 18 hours after implantation of the dialysis probe, basal extracellular DA levels were determined at 15 minutes intervals (all data expressed as pg/20 uL). Microdialysis probes (60 ± 5% DA and 24 ± 7% 5-HT) placed lateral to the mPFC, basal levels of DA in shell were increased relative to core controls (7.2 ± 0.6 (n=6) and 5.0 ± 0.7 (n=8), respectively). In contrast, basal DA levels were not different in core of lesioned and control rats (9.0 ± 1.0 (n=5) and 8.4 ± 0.7 (n=6), respectively). Systemic d-amphetamine (1.5 mg/kg, p.o.) produced a similar increase of extracellular DA in shell of both lesioned and control rats (28 ± 3.1 and 29.0 ± 4.0, respectively). In contrast, mPFC lesions attenuated the effects of d-amphetamine on DA release. The ability of 6-OHDA lesions to produce a DA lesion was confirmed by analyzing DA content, which was 8.5 ± 0.8, respectively. The previous data suggest that the DA neurons contained within the mPFC projects to the nucleus accumbens shell. Further investigation is required to determine if the DA neurons projecting to the shell are differentially regulated under conditions of stress. [Supported by Tourette Syndrome Association, Scottish Rae Schizophrenia Research Program, National Alliance for Research on Schizophrenia and Depression and USPS grants MH45156 and MH43947.]

639.10

The present study examined the role of glutamate in the regulation of stress-evoked changes in tyrosine hydroxylase (TH) in neostriatum. Adult male rats were exposed to 30 min of intermittent tail shock in the presence of an amino acid decarboxylase (AAAD) inhibitor, NSD-1015 (100 µM), administered locally through a dialysis probe implanted into the striatum, and in the concurrent control of DopA in the dialysate was measured. Stress was applied beginning either 15 min or 75 min after the onset of NSD treatment, corresponding to the initial rate or steady-state phase of the DOPA accumulation rate, respectively. Stress was administered before the steady-state levels of extracellular DOPA (+40%). How ever, no change was observed in the initial rate of DOPA accumulation rate after the stress was administered, but the steady-state levels of extracellular DOPA (+40%). Similar results were obtained using APV (100 µM) or CNQX (100 µM), glutamate antagonists. Glutamate potentiated the time course of stress-evoked increase in extracellular DA in shell compared to controls (8.5 ± 0.8 and 5.9 ± 0.8, respectively). These data suggest that the mesoaccumbens DA neurons functioning in shell but not core are regulated by sympatoadrenal and corticosteroids evoked response, under conditions of stress. [Supported by Tourette Syndrome Association, Scottish Rae Schizophrenia Research Program, National Alliance for Research on Schizophrenia and Depression and USPS grants MH45156 and MH43947.]

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639.11

DOPAMINE-SOMATOSTATIN INTERACTIONS IN THE RAT STRIATUM. J.R. Blackburn1, C.A. Chapman1, C.D. Blaha2 and J.S. Yeomans, Dept. of Psychology, Simon Fraser University, Burnaby, British Columbia, Canada.

The neuropeptide somatostatin is found throughout the central nervous system and is believed to act as a neurotransmitter. Pharmacological and behavioral evidence suggests a role for somatostatin in dopamine-mediated behaviors. To assess such an interaction between the two, the authors examined the effect of somatostatin on dopamine release, using in vivo microdialysis. Apomorphine administered subcutaneously, and directly infused into the striatum, did not affect somatostatin release, but was used for the infusion of somatostatin, as well as the collection of the dialysate at 15 min intervals (1 uL/min). Dopamine and metabolites were measured prior to and after administration of somatostatin. The results indicate that a striatal interaction exists between somatostatin and dopamine, in the striatum, at the neurochemical level.


639.13

NEUROKININ-1 TACHYKININ RECEPTOR-DOPAMINE INTERACTIONS IN THE RAT. M. Blackstone, C.J. Gibson1 and A.A. Stossel, Clinical Neurological Sciences, University of Western Ontario, London, ON, Canada, N6A 5A5.

There is anatomical evidence for a dense substance P (SP) innervation from the substantia nigra (SN). Electrophysiological, neurochemical and behavioral studies all suggest a functionally significant interaction between tachykinins and dopamine (DA) neuron and the SN, but this structure is devoid of SP receptors. Pharmacological and molecular studies have demonstrated that SP and related compounds act through a family of tachykinin receptors, termed neurokinin (NK) receptors. Autoradiographic studies have demonstrated the presence of NK-3 receptors on nigral DA neurons and stimulation of nigral NK-3 receptors leads to decreases in DA-mediated behavioral responses and locomotion and rearing. In contrast, stimulation of nigral NK-1 receptors elicits grooming in the absence of locomotion and rearing. We explored the possibility that NK-1-induced grooming is a response to the release of a substance, which would then stimulate local DA D1 receptors. Nigral infusion of the selective NK-1 agonist (Ac-Arg4, Sar6, Met7) [Leu] Phe9 (0-1 mmol) elicited dose-dependent stereotypic grooming. This response was significantly attenuated by peripheral administration of the DA D1 antagonist SCH23390 (100 μg/kg s.c.), but not by the DA D2 antagonist sulpiride (30 mg/kg i.p.). Grooming induced by nigral infusion of the NK-1 agonist was unaffected by either nigral or striatal SCH23390 (0.1-5 μg), but was blocked by bilateral 6-hydroxydopamine lesions. Our findings indicate that stimulation of nigral NK-1 receptors elicits grooming which is dependent upon release of DA and stimulation of D1 receptors in an as yet unidentified site.

639.15

STRIATAL DOPAMINE LEVELS INCREASE FOLLOWING PERIPHERAL SCOPOLAMINE INJECTIONS AND FOLLOWING APPLICATION OF SCOPOLAMINE TO THE TEGMENTAL PEDUNCULOPONTINE NUCLEUS. J.R. Blackburn1, A. Chapman, C.D. Blaha and J.S. Yeomans, Dept. of Psychology, Simon Fraser University, Burnaby, British Columbia, Canada.

The cholinergic cells of the tegmental pedunculopontine nucleus (PPT) monosynaptically excite dopaminergic (DA) neurons of the substantia nigra. Furthermore, DA-dependent behaviors are modulated by both peripheral or central PPT injections of antimuscarinic drugs, consistent with a disinhibition of PPT cholinergic cells via blockade of their inhibitory autacoid, acetylcholine. Rat dorso-lateral striatal DA levels were monitored chronocronoamperometrically (1sec 550mV pulses) with bilateral, chronically implanted, steelate-modified graphite paste electrodes used to inject scopolamine (1.25mg/kg i.p.) into the PPT. Injections of scopolamine into the PPT (10, 50, 100μg/side) or into the striatum (100 μg) resulted in bilateral dose-related increases in DA oxidation currents in the striatum. Unilateral microinjections of scopolamine into the PPT (10, 50, 100 μg/side) also resulted in short-latency, bilateral dose-related increases in striatal DA oxidation currents which usually peaked 50-80 min post-injection. Cholinergic PPT 20 min prior to 100μg PPT scopolamine attenuated these increases. These results indicate that elevated striatal DA levels following the systemic scopolamine treatments may be mediated by the PPT. This study further demonstrates a powerful excitatory effect of Ch5 PPT cells on the nigrostrial DA system.

639.16

CHRONIC DOPAMINE TREATMENT ALTERS THYMOID BINDING TO STRIATAL NMDA RECEPTORS AND DOPAMINE UPTAKE SITES IN THE RAT. J. Dell1, F. Wehlmuller2, J.F. Marshall2, Dept. of Psychology, University of California, Irvine, CA 92717.

We have previously shown that chronic haloperidol treatment increases binding to NMDA receptors in the outer layers of parietal cortex. To further elucidate this phenomenon, we investigated the effect of long-term (3 weeks) blockade of dopamine D2 or D1 receptors (both or both) on binding to NMDA receptors in rat hippocampus and striatum. [3H]Glutamate (5.0 mg/kg i.c.e., 75 μCi) and [3H]Oligo (10.0 mg/kg/day, i.p.) were administered to groups of rats. H-defactio (200 μg/day, s.c.) was used as a positive control. Our results indicate that chronic haloperidol treatment increases binding to NMDA receptors in the outer layers of parietal cortex, and the pyramidal layers of the hippocampus, while significantly attenuates binding to NMDA receptors in the lateral and ventral caudate-putamen, and nucleus accumbens. [3H]Oligo alone generally had no effect on NMDA receptor binding. The other hand, simultaneous blockade of D1 and D2 receptors resulted in a slightly smaller increase (than that evoked by scopolamine alone) in binding to NMDA receptors in the frontal cortex, an increased binding in the deep layers of parietal cortex and CA3 region, and further reduced binding to NMDA receptors in the striatum. These results suggest that D2 dopamine modulates NMDA receptor function differently in basolateral VS dorsal cortical regions, D2 although SCH23390 alone does not affect binding to NMDA receptors, chronic blockade of D1 receptor may modify the effects of D2 receptor inhibition, and if so the effect of haloperidol on NMDA receptors cannot be completely explained by blockade of D2 receptors.
639.17

NEUROTRANSMITTER INTERACTIONS


The distribution of NADPH-diaphorase activity (ND), an enzyme originally identified as the nitric oxide synthase, was histochemically investigated in the Japanese quail brain. Two levels of positive neurons were observed at all levels of the brainstem. In particular, they were located within the substantia nigra (SN), the area ventralis tegmentalis (AVT), the substantia grisea centralis, the nucleus interpeduncularis (LOC) and the subcoerulean (SCo). Scattered elements were present in other regions such as the vestibular nuclei or the nucleus of the solitary tract (S). This distribution partly overlaps with catecholamine (CA) neurons and with histochemical and immunohistochemical techniques. In most mammalian species, ND-positive neurons also show immunoreactivity for choline acetyltransferase (ChAT) and are intermingled with other histamine neurons. In the present study, we performed a double staining for ND and ChAT (anterior part of the mesencephalon and mesopontine region). At the level of midbrain, a large number of CA-positive neurons was also evident in both brainstem areas and in the midbrain. These results indicate that the mesopontine ND systems, is to identify neuronal populations containing different neurotransmitters (N), the demonstration that the great number of immunopositive neurons of SN and AVT are largely positive for ND suggests that the nitric oxide could have an important role in regulating the target areas and neurons in the brainstem. Supported by grants of CNR and MURST 60%.

639.18

NEUROPEPTIDE Y INCREASES CLOW INTAKE AND DOPAMINE CONCENTRATIONS IN MICRODIALYSATE FROM THE NUCLEUS ACCUMBENS OF RATS IN A FOOD CHOICE PARADIGM. R.L. Corwin* and J.L. Crawley, SBN, ETO, NIH, NH, Building 10, Room 4W212, Bethesda, MD 20892.

Neuropeptide Y (NPY) has been shown to be the most prevalent immunoreactivity in the brainstem (Hattori, Neuroni Res, 1993; similarly, serotonephine receptors appear to release glutamate (Johnson, Neuroni, 1994). Electrophysiologically studies of single identified neurons show that the cells frequently make glutamatergic autaptic EPSPS (Sulzer and Rayport, Soc Neurosci Abs, 1993). We now report that individual dopamine neurons immunostained for tyrosine hydroxylase show significant variation in neurite staining. In single neuron microcultures, where all processes arise from a single dopamine neuron, some thin neurites — which are most likely axonal — have varicosities that stain darkly and others have varicosities that stain lightly or are unstained. Double staining for synaptophysin shows putative release sites that do not stain for tyrosine hydroxylase. Double immunostaining with a polyclonal antiserum directed against glutamate (the glutaraldehyde-conjugate) shows that dopamine neurons are often glutamate-positive, while GABA neurons are rarely glutamate-positive. Taken together, these observations are consistent with the possibility that dopamine neurons release glutamate as a cotransmitter and may do so at separate terminals from where they release dopamine. If so, this may account in part for glutamate dependence of dopamine synaptic plasticity and neurotoxicity.

639.19

NEUROPEPTIDE Y RELEASE FROM PC12 CELLS IS REGULATED BY DOPAMINE X. Chen*, S.P. Han and T.C. Westfall Department of Pharmacological and Physiological Science, St. Louis University Health Science Center, St. Louis, MO 63104.

We have previously demonstrated that NPY is co-localized and co-released with dopamine from phenotypically PC12 cells by potassium-induced depolarization (J. Neurochem. Vol. 61, suppl., 1993). This study was designed to further investigate the mechanisms regulating NPY release from the peripheral sympathetic nervous system using PC12 cell line as a model system. PC12 cells grown in cell culture were partially differentiated with NGF for 3-4 days. NPY content in the cells and in the releasing buffer was determined by radioimmunoassay as described previously (DiMaggio, 1992). KCI (50 mM) induced a 1-2 fold increase in NPY release over basal levels. KCl-stimulated NPY release was greatly attenuated by the activation of dopamine receptors on PC12 cells with apomorphine, a non-selective D1 and D2 dopamine receptor agonist. The inhibitory effect of apomorphine on NPY release was dose dependent and could be partially reversed by eticlopride, a D2 dopamine receptor selective antagonist.

These results suggest that NPY release from PC12 cells is regulated by dopamine which acts as a cotransmitter of NPY in this model of a peripheral sympathetic neuron. This work is supported by HL26319, HL35202 and NS02254.

639.20


Haloperidol and clozapine are two drugs that decrease extracellular dopamine concentration in the brain and have potential therapeutic use in the treatment of drug abuse. In these experiments, we evaluated the effects of these drugs on extracellular dopamine concentrations in the nucleus accumbens (NA) of the rat. Haloperidol decreased extracellular dopamine concentrations by approximately 30% whereas clozapine was ineffective at doses of up to 10 mg/kg. We have previously demonstrated that NPY is present in the NA at high concentrations. NPY has been shown to be important in the regulation of dopamine release. NPY has been shown to inhibit dopamine release by binding to neurotensin (NT) receptor. The interaction between NPY and NT receptors may be important in the regulation of dopamine release. In the present study, we tested these hypotheses by using hPLC-EC. NPY inhibited dopamine release and dopamine concentration, while having no effect on dopamine release in the NPY receptor activity. These results suggest that NPY may play a role in food choice and that this effect may involve mesolimbic DA. 1. Clark, et al., 1994, Endocrinology 115:427. 2. De Quist and Emson, 1986, Neuroscience 18: 545. 3. Salin, et al., 1990, Exp. Brain Res. 81:363. 4. Aoki and Pickel, 1988, Brain Research 459:205.

640.1


The distribution of NADPH-diaphorase activity (ND), an enzyme originally identified as the nitric oxide synthase, was histochemically investigated in the Japanese quail brain. Two levels of positive neurons were observed at all levels of the brainstem. In particular, they were located within the substantia nigra (SN), the area ventralis tegmentalis (AVT), the substantia grisea centralis, the nucleus interpeduncularis (LOC) and the subcoerulean (SCo). Scattered elements were present in other regions such as the vestibular nuclei or the nucleus of the solitary tract (S). This distribution partly overlaps with catecholamine (CA) neurons and with histochemical and immunohistochemical techniques. In most mammalian species, ND-positive neurons also show immunoreactivity for choline acetyltransferase (ChAT) and are intermingled with other histamine neurons. In the present study, we performed a double staining for ND and ChAT (anterior part of the mesencephalon and mesopontine region). At the level of midbrain, a large number of CA-positive neurons was also evident in both brainstem areas and in the midbrain. These results indicate that the mesopontine ND systems, is to identify neuronal populations containing different neurotransmitters (N), the demonstration that the great number of immunopositive neurons of SN and AVT are largely positive for ND suggests that the nitric oxide could have an important role in regulating the target areas and neurons in the brainstem. Supported by grants of CNR and MURST 60%.

640.2

SIMULTANEOUS VOLTAMMETRIC DETECTION OF STIMULATED DOPAMINE AND NITRIC OXIDE RELEASE IN RAT BRAIN SLICES: M.Iravani, J. Millar & E.L. Kruks, Departments of Pharmacology and Physiology (JM), Queen Mary & Westfield College, Mile End Rd, London E1 4NS UK.

We have recently described a voltammetric method which uses a carbon fibre electrode for real time detection of nitric oxide (NO; Iravani et al 1993, J. Physiol., 496: 48P), and we have used this to examine a NO like signal in rat striatum. Pressure ejection of NMDA, or electrical stimulation with trains of pulses in a 350µm rat striatum slice, gave rise to complex voltammetric signals which could be resolved into components attributable to the presence of dopamine (DA) and NO. The transients for the DA and NO signals were different. The signal attributed to NO could be reduced by addition of the NOS inhibitor L-NAME to the perfusion fluid. In the presence of the endogenous NO like substance, release of DA by electrical stimulation was attenuated, indicating that NO may have a role in local regulation of dopamine release.

Glutamate activation of NMDA receptor ion channels and the subsequent influx of Ca\(^{2+}\) is known to result in the activation of nitric oxide synthase (NOS) and enhanced nitric oxide (NO) production. While glutamate and NO are known to be involved in the excitotoxic process of neuronal degeneration the exact mechanisms of this process are still unclear. The objective of this research was a first step towards the goal of better elucidation of the role of these transmitters in the excitotoxic process. This initial step entails the application of iontophoretic application methods to the evaluation of glutamate and NO overflow in the extracellular fluid (ECF) of the rat hippocampus. Evoked glutamate overflow was monitored using a dual enzyme biosensor developed in our laboratory. NO was measured using a nickel peroxidase based sensor developed by Malinski and colleagues (Nature 358:676-678, 1993). Glutamate and NO overflow were evoked by the local injection of depolarizing levels of K\(^+\) into the hippocampus. Potassium-evoked glutamate release was correlated with local NO production. Local administration of nitro-L-arginine methyl ester (L-NMAE), an inhibitor of NOS, resulted in a decrease in NO production as well as a decrease in glutamate overflow. Furthermore, application of L-arginine in addition to L-NAME reversed this effect. Our results indicate that NO and glutamate in the ECF can be detected simultaneously in vivo using electrochemical detection methods. Last, we observed in the enhanced overflow of glutamate in confirmation of similar findings reported previously (Buisson, et al. J. Neurochem. 61:990-996, 1993).

640.4 NITRIC OXIDE INHIBITS NEUROGENIC NON-ADRENERGIC, NON-CHOLINERGIC CONTRACTIONS BUT NOT CHOLINERGIC CONTRACTIONS OF CIRCULAR MUSCLE FROM GUINEA PIG ILEUM. J.J. Gallagher, and A.M. Yunker. Dept. Pharmacology and Toxicology and Neuroscience Program, Michigan State University, E. Lansing, MI 48824.

Nitric oxide (NO) is released from enteric neurons resulting in relaxation of gastrointestinal (GI) smooth muscle. NO may also inhibit release of excitatory neurotransmitters such as substance P (SP) and acetylcholine (ACh) from enteric nerves. This hypothesis was tested using circular muscle-myenteric plexus preparations (CMMPs) isolated from guinea pig ileum in the presence of scopolamine and guanethidin, stimulation (1-50 Hz, 0.1 Ts) of CMMPs produced non-adrenergic, non-cholinergic (NANC) contractions. These contractions were blocked by tetraethylammonium and attenuated in a concentration-dependent manner by CP96345-1, a neurokinin-1 receptor antagonist. The nitric oxide synthase (NOS) antagonists N\textsubscript{n} -nitro-L-Larginine, N\textsubscript{n} -nitro-L-arginine methyl ester, and N\textsubscript{n} -methyl-L-arginine potentiated the amplitude of the NANC contractions in a concentration-dependent manner. The D stereoisomers of these NOS antagonists did not alter the NANC contractions. Onychomycosis binds excitatory neurotransmitters and caused a concentration-dependent increase in the amplitude of NANC contractions. Neither the NOS antagonists nor onychomycosis altered the contraction-response curve for substance P methyl ester, suggesting that it is not the new receptor responsible for these drugs. Finally, sodium nitroprusside attenuated NANC contractions in a concentration-dependent manner. Neither the NOS antagonists nor onychomycosis affected cholinergic contractions generated in the presence of guanethidin and CP96345-1. These data suggest that NO inhibits SP but not ACh release, thus providing a second mechanism by which NO can inhibit motility of GI smooth muscle. (Supported by DK 40120 and NS 07279).

640.5 NITRIC OXIDE DEPOLARIZES PARAVASCULAR NUCLEUS NEURONS IN VITRO. J.S. Bains* and A.V. Ferguson. Department of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

The presence of nitric oxide synthase, the enzyme necessary for the production of nitric oxide (NO), within neurons in the paravascular nucleus of the hypothalamus (PVN) has led to the suggestion that NO may play a role in neurotransmission within this structure. Recent in vivo experiments have demonstrated that diazoxide of NO directly into PVN everts decreases in blood pressure. This physiological change is accompanied by an increase in the release of excitatory amino acids such as glutamate within this nucleus (Horn et al., AJP, 1994). In this study, we have used whole-cell patch clamp recording techniques to investigate the potential effects of NO on PVN neurons. Using patch electrodes filled with potassium-glucose, recordings were made from PVN neurons in coronal brain slices (400 \mu m thickness) of adult, male Sprague-Dawley rats. Bath application of NO (10^\text{-8} \text{ M}, 10^\text{-9} \text{ M}) induced a hyperpolarisation (1.2 mV to 17.9 mV) membrane depolarization in 6 of the 7 cells tested (mean depolarization \pm SEM: 10.6 \pm 4.1 mV). This NO induced depolarization was also accompanied by a mean decrease in membrane resistance of 28 \pm 17 \%.

These results indicate that NO, either itself, or through influencing the release of other neural mediators acts to excite PVN neurons.

640.6 EFFECTS OF NITRIC OXIDE ON NITRIC OXIDE AND NEUROTRANSMITTER RELEASE IN THE RAT STRIATUM. B. Guevara-Guzman, P.C. Ferguson, and K.M. Kreider. Dept. Neurobiology, BSSRC Bahadur Institute, Cambridge CR4 4AT, U.K. Dept. Physiology, Faculty of Medicine, Mexico University, Mexico City 04510, DF.

We have shown previously that nitric oxide (NO) stimulates in vivo release of acetylcholine (ACh), 5HT, glutamate (Glu), inotrine (TAU) and GABA in the rat striatum and that these effects are mimicked by 

\[ \text{NO} \rightarrow \text{NO} + \text{O}_2 \] \[ \text{NO} + \text{O}_2 \rightarrow \text{NO}_2 \text{O}^+ \] \[ \text{NO}_2 \text{O}^+ + \text{H}^+ \rightarrow \text{NO}_2 + \text{H}_2 \text{O} \] \[ \text{NO}_2 + \text{H}_2 \text{O} \rightarrow \text{NO} + \text{H}_2 \text{O}_2 \] \[ \text{NO}_2 + \text{H}_2 \text{O} \rightarrow \text{NO} + \text{H}_2 \text{O}_2 \] \[ \text{NO}_2 + \text{H}_2 \text{O} \rightarrow \text{NO} + \text{H}_2 \text{O}_2 \] \[ \text{NO}_2 + \text{H}_2 \text{O} \rightarrow \text{NO} + \text{H}_2 \text{O}_2 \] \[ \text{NO}_2 + \text{H}_2 \text{O} \rightarrow \text{NO} + \text{H}_2 \text{O}_2 \] \[ \text{NO}_2 + \text{H}_2 \text{O} \rightarrow \text{NO} + \text{H}_2 \text{O}_2 \] 

These reactions are accompanied by a significant increase in NO production. However, NO release was not detected using NO-sensitive electrodes. The experiments were conducted since NO is not a neurotransmitter and in most cases NO release was detected in the striatum, although inhibiting NO release with NAGB potentiates this release. These results provide further support for a nonneuronal role for NO. The fact that NOFMCS may have other sources which are not neurotransmitter sources, in order to release NO in the CNS, it may be more appropriate to use in the CNS. We have investigated the following: a) the presence of NO without the use of NAGB and b) the presence of NO without the use of NAGB and c) the presence of NO without the use of NAGB and d) the presence of NO without the use of NAGB and e) the presence of NO without the use of NAGB and f) the presence of NO without the use of NAGB and g) the presence of NO without the use of NAGB and h) the presence of NO without the use of NAGB and i) the presence of NO without the use of NAGB and j) the presence of NO without the use of NAGB and k) the presence of NO without the use of NAGB and l) the presence of NO without the use of NAGB and m) the presence of NO without the use of NAGB and n) the presence of NO without the use of NAGB and o) the presence of NO without the use of NAGB and p) the presence of NO without the use of NAGB and q) the presence of NO without the use of NAGB and r) the presence of NO without the use of NAGB and s) the presence of NO without the use of NAGB and t) the presence of NO without the use of NAGB and u) the presence of NO without the use of NAGB and v) the presence of NO without the use of NAGB and w) the presence of NO without the use of NAGB and x) the presence of NO without the use of NAGB and y) the presence of NO without the use of NAGB and z) the presence of NO without the use of NAGB.
640.11 MUSCARIC MODULATION OF NMDA RESPONSES IN NEOCORTEX. V. B. Aramazyan1, J. H. Ashe and A. E. Bandrowski. Deps. of Neuroscience and Psychology, Univ. of California, Riverside, CA 92521.

Muscarinic actions of acetylcholine facilitate the amplitude of glutamate-induced membrane depolarizations (Ds) in rat auditory cortex (Cox et al, Synapse, 16;123, 1994). To assess the contribution of NMDA receptors to this facilitation, the actions of 1nM (-)-methylscopolamine (MCh) were tested upon glutamate-induced Ds elicited in the presence of the AMPA/kainate antagonists CNQX or NBQX (20 µM). The actions of MCh upon the isolated Ds (MCh preconditioned) were also examined. Whole-cell recordings were obtained from layer III cortical neurons of the in vitro auditory cortex. Isotophoretic application of MCh (1M, 50-100 nA, 1.5 min) or glutamate (1M, 500 nA, 20 sec) was also applied to layer III within 50 µm of the recording electrode. During MCh, the amplitudes of glutamate Ds were consistently suppressed in either the presence or absence of CNQX or NBQX. On the other hand, following MCh, the amplitudes of glutamate Ds were largely facilitated; about 75% in the absence of CN/NBQX, and about 300% in the presence of CN/NBQX. Durations of facilitation could last up to 35 min. MCh was also tested upon the isolated late-EPSs, i.e., elicited in the presence of CN/NBQX at 10-20 µM to block AMPA/kainate receptors, and to also minimize the contribution of the IPSPs (Methaure & Ashe, in press). Under these conditions, the isolated late-EPS was consistent suppressed by MCh. Moreover, the isolated late-EPS amplitude was usually enhanced above pre-MCh levels, following the initial suppression. The actions of MCh on glutamate-induced Ds and the isolated late-EPS suggests an involvement of NMDA receptors in cholinergic modulation of glutamatergic transmission. Supported by NSF (IBN 1010822).

640.12 EFFECT OF NMDA RECEPTOR BLOCKADE ON REGIONAL DOPAMINERGIC ACTIVITY. G. A. Fayed, V. J. Taylor, Geoffrey Metcalfe and Christopher J. S. Cornish. Marion Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45252.

A dopaminergic bias within the glutamate/dopamine (DA) balance of the basal ganglia is believed to contribute to the symptoms of schizophrenia. The psychotomimetic MK-801, a channel blocker at the NMDA/cholinergic agonist receptor complex, is used to model this imbalance by reducing glutamate activation of NMDA receptors. Although MK-801 can produce its behavioral effects in monosodium-denervated animals, this occurs at higher doses than are necessary to elicit behavior in intact animals. MK-801 is also reported to increase the firing rate and bursting pattern of midbrain DA neurones. These observations suggest that increases in DA activity as well as decreased glutamatergic activity may contribute to the behavioral effects of MK-801. To further explore this question, MK-801 was administered to rats and its effect on DA release was determined in several brain regions using microdialysis in awake, freely moving rats. High doses of MK-801 (2 mg/kg, i.c.v.) caused a rapid increase in extracellular concentrations of DA in both the midbrain (VTA, SN) and the nucleus accumbens (NAS). DA concentrations reached 30% of basal levels in the NAS at 20 min after injection, and increased to approximately 150% in the NAS. Transmitter levels remained elevated for 3 h. Concentrations of the DA metabolite, homovanillic acid (HVA), also increased in all regions, suggesting that MK-801 also increased dopamine synthesis in both regions as assessed by DOPA metabolism prior to deacetylation inhibition. In contrast to the results in the NAS and VTA, striatal DA and its metabolites decreased following MK-801 administration. These observations are consistent with the hypothesis that alterations in DA activity contribute to the behavioral effects of MK-801. The results also suggest that the mesolimbic and mesostriatal DA systems differ significantly in their interaction with the NMDA glutamate system.


In our previous study, noradrenaline was found to be able to synergistically enhance glutamate induced intracellular free calcium ([Ca2+]i) increases in cultured visceral cortical neurons (Neurosci. Abst. 205.13,1993). To further address the mechanism underlying this interaction, we employed antagonists of receptor subtypes to investigate the cellular pathways responsible for the modifying effect. We found that the dose dependent glutamate induced calcium increase was blocked by 25 µM APV. It was interesting to find that this NMDA receptor antagonist also completely blocked the synergistic increase in [Ca2+]i, observed when noradrenaline and noradrenaline were coupled. This result indicates that the noradrenaline enhanced increase in calcium requires at least an initial activation of NMDA receptor channels. Further possible sources of calcium mobilisation include facilitated NMDA receptor channels, voltage gated calcium channels, internal calcium stores or any combination of these potential routes. We also tested three major subtypes of adrenergic receptors with respective antagonists, propranolol (B), phenolamine and yohimbine (Y). Since we were dealing with a mixed culture of cortical neurons, these antagonists showed varying effectiveness in blocking the synergistic increase induced by 10-5 individual neurons. Analyses of data obtained thus far indicate that propranolol is capable of blocking the synergism more completely on more cells than yohimbine. Phenolamine showed relatively weak effects. This implies that both AMPA and PI turnover pathways may be involved in the synergistic interaction between these two factors. Further studies being carried out by manipulating intracellular cascades that may be involved.


The distribution of myomodulin-like immunoreactivity in the leech CNS was determined using an antisem raised against Aplysia myomodulin (Miller et al., 1991). Numerous immunoreactive neurons were found in the nervous system. Also, immunoreactive varicosities were found throughout the neuropil. Two types of immunoreactive fiber bundles were observed in the connectives between ganglia and in the nerve roots. 1) fibers with smooth contours and 2) fibers with periodic swellings. Double-labeling experiments are in progress to identify the labeled neurons. Preliminary results suggest that myomodulin-like peptides are present in the anterior and posterior segments of the leech. Myomodulin-like peptides and the light/dark cell, components of the shortening reflex which can be modified by learning. Kuhlman et al., (1985) have previously demonstrated FMRF-like-reactivity in these cell types as well. Specific staining was abolished by preabsorption of antisem with synthetic myomodulin but not with FMRF-amide. It is likely, therefore, that these cells contain multiple neuropeptides. The immunostaining profile data are consistent with the presence of myomodulin in the leech CNS. Supported by R01MH44789.
641.1

HYPOTHYROIDISM IMPROVES THE STRESS RESPONSE AND MODIFIES ANXIETY BEHAVIOR IN RATS.
S. L. Aboud*, J. A. Lindsay, D. Brown, J. A. King, L. Feltien, C. Eckerl, and E. Edwards*

Learned Helplessness in the LH, an animal model that determines the response of rats to footshock stress, has been used to screen pharmacologic agents for use in depression. In addition to tricyclic antidepressives, low dose T3 administration and GABA-aminergic agents have been shown to inhibit the behavioral response in this paradigm. Low doses of T3 may actually cause brain hypothyroidism by decreasing pituitary secretion of thyrotopin, thereby inhibiting endogenous thyroid hormone secretion. We tested this hypothesis by making hypothyroid rats for LH. Hypothyroid rats had a 25% lower incidence of LH and a 20% increase in LH-resistant behavior compared to euthyroid controls (p<0.05). There were no differences in activity level by open-field tests, nor in escape distance or number of entries into the LH. Hypothyroid rats also displayed anxiolytic behavior relative to euthyroid rats in the elevated plus maze, a behavioral assessment of GABAergic activity, although on testing 1 month later, hypothyroid LH rats displayed anxiolytic activity. Hypothyroid rats did not display differences in GABA receptor sensitivity compared to euthyroid controls, as assessed by GABA, GABA receptor-mediated chloride ion influx. Euthyroid-LH-resistant rats did manifest an increase in GABA, receptor sensitivity compared to euthyroid LH controls. We conclude from these studies that hypothyroidism decreases the incidence of LH behavior, and the antidepressant activity of T3 may actually be due to its induction of secondary brain hypothyroidism. This effect may be mediated in part by a hypothyroidism-induced enhancement of GABAergic transmission by a mechanism other than GABA receptor sensitivity.

641.3

DOES SEASONAL VARIATION AFFECT RESULTS OF THE MIRRORED CHAMBER BEHAVIORAL ASSAY? S. J. Ciric and N. L. Katz*
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We studied several mirrored chambers described by Toubas et al. (Pharmacol. Biochem. Behav. 35: 121, 1990). The mirrored chamber test is a behavioral assay based on an approach-avoidance response when a mirror is placed into a mouse's environment. The sight of an animal reflected in the mirror is a source of anxiety presumably preventing entry into the chamber. In the present studies, male Balb/cByJ mice were used. Their ages, at the onset of the studies were 7-8 weeks. Each mouse was allowed to explore the chamber for 5 min. The time to first entry into the mirrored chamber (T), total time spent in the chamber (TT), and number of entries (NE) were quantitated. Control studies were run during the first 2 weeks of October (1993), first 2 weeks of December, first 2 weeks of February (1994), and last week of April. Latencies to enter were 182 (N=30), 102 (N=47), 107 (N=17), and 236 sec (N=10), respectively. In the winter months, results seemed heterogeneous and inconsistent, and base line (percent of animals entering the chamber) ranged from 50-75%. During this season, cholecystokinin tetrapeptide (CCK), in doses ranging from 3-30 mg/kg, produced a dose-dependent increase in T, and decreased both TT and NE. The CCK antagonist PD135158, 1 mg/kg, reversed CCK-induced changes in T and TT. PD135158 alone was without effect. Results obtained in April were most similar to those reported by Toubas et al. (1993). Diazepam, 1 mg/kg, decreased T, and increased TT and NE. PD135158 had no effect.

641.5

ESTABLISHMENT OF MELATONIN DISCRIMINATION IN PIGEONS. J. A. Stanley* and A. A. Ortiz
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Melatonin (5-methoxy-N-acetyltryptamine) is a pineal neurohormone that controls circadian patterns of behavior (e.g., sleeping, eating, drinking, and reproduction) in many species by modulating the “biological clock,” a cluster of cells found in the suprachiasmatic nucleus (SCN). In order to permit exploration of the functional effects of melatonin, pigeons were trained to use the interoceptive cue produced by peripheral administration of melatonin as a discriminative stimulus for differential responding using a drug discrimination procedure. Ten minutes before daily fifteen-minute sessions, White Carneau pigeons were given melatonin (0.2 or 0.56 mg/kg, i.m.) or vehicle (0.5% DMSO in normal saline) and placed in a darkened, two-key operant chamber. Responding on the appropriate key was reinforced (FR-30); whereas, responding on the non-reinforced key was time-out. Performance was monitored on the basis of more than resetting the FR value.

Pigeons learn to discriminate a systematically administered dose of melatonin from its vehicle at doses that have no effect on response rate. When doses of melatonin from 0.01 and 3.0 mg/kg, i.m., are substituted for the training dose (0.2 or 0.56 mg/kg, i.m.), the drug-key is selected even if there is no time-out performance on the other key.

In rats melatonin discrimination has been demonstrated only at doses which are sedative and more than 100 times those used in circadian rhythm studies. If the requisite sensitivity of the pigeon to melatonin, suggest the bird may be the subject of choice in discrimination studies investigating the effects of melatonin at doses comparable to those used in circadian rhythm studies.

641.6

EFFECTS OF THE CARBAMATE PESTICIDE CARBARYL ON THE REPEATED ACQUISITION OF RESPONSE SEQUENCES IN RATS. J. Croll and R. C. MacPhail*
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Carbaryl is a commonly used pesticide that inhibits acetylcholin-esterase. Cholinergic neurotransmission is known to play an important role in cognitive function. This experiment was therefore undertaken to determine the effects of acute carbaryl administration on the repeated acquisition of response sequences in rats. Repeated acquisition is a method by which learning can be continuously assessed in single subjects. Adult male Long- Evans rats, maintained at 300 g performed under a multiple schedule of RA and performance (P) in a standard operant chamber equipped with three response levers. The RA component required rats to learn a new 4-member sequence of responses during each experiment session (Center Right Left Right, RLCL, KCRL, etc.). The correct sequence of responses for the P component remained constant across sessions. Components alternated twice during a session. After baseline training, rats were given vehicle (5% Emulphor, 5% ethanol in saline), 3, 6, 7.5, 10 and 17.5 mg/kg carbaryl. Injections were given i.p. (1 ml/kg) 20 min preinjection, and no more than twice weekly. Significant decreases in both accuracy and response rate were produced by doses of 6.5 mg/kg and greater. Generally, results indicated that carbaryl’s effects on RA were non-specific in that both the RA and P components were equally affected. Some rats, however, exhibited specific effects of carbaryl on RA. Microanalysis of response patterning indicated that errors consisted primarily of pseudoreversals (skiing over errors) rather than perseverative responses on single levers.

641.4

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Gamma-hydroxybutyrate (GHB) is an endogenous fatty acid recognized for its sedative-hypnotic effect. Since thermoregulatory mechanisms are bound to be involved in this effect, we measured central temperature before and after administration of GHB at various doses and we tested the newly synthesized GHB receptor antagonist, NCS-382. Body temperature was monitored by mean of a rectal probe; baseline readings were first taken, then each 15 min for the 1st hour following an i.p. injection of saline or GHB, and each hour thereafter. When NCS-382 was used, it was co-administered with GHB, at the same dose. Low GHB doses (10 mg/kg) were associated with increased temperature (max = +0.67°C, 45 min post injection) whereas hypothermia was observed from 20 to 252 mg/kg (max at 250 mg/kg = -0.95°C, 30 min post injection). NCS-382 blocked the hyperthermic effect of GHB 10mg/kg but not the hypothermia induced by GHB 250mg/kg. These results suggest that the pyrogenic effect of GHB is mediated by high affinity specific GHB receptors and that GHB-induced hypothermia involves a more complex mechanism. Supported by FRGS and CNRS.

641.6

RESTORATIVE EFFECTS OF FIBROLAST GROWTH FACTOR, GIVEN TEN DAYS AFTER FIBROMIA-FORNIX SEVERANCE, ON SPATIAL MEMORY IMPAIRMENT IN RATS. H. Amin*, N.Suzuki and H. Matsuzawa
Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113, Japan.

Fibroblast (F-F) is a major collagenolytic afferent from medial septum to hippocampus, which is suggested to play a critical role in spatial learning. We have previously reported that both acute and chronic fibroblast growth factor (a bFGF) accelerated the acquisition and reversal learning in Y-maze test in naive rats. In the present study, we studied the effects of bFGF on spatial memory deficits induced by F-F lesion. Rats with a normal spatial learning ability had been selected, based on the results of Y-maze test. Guidance cannula were planted into bilateral coroebroventriculi, and F-F was severed at a blade at the same time. From 10 days after the operation, spatial learning performances of the rats were observed in Y-maze test, and subsequently in water maze test. FGFS (100-400 ng/rat, i.c.v.) were daily injected 30 min prior to each learning session. F-F lesion induced severe and long-lasting spatial memory deficits in Y-maze test (correct response: intact 96.5±8.2 %, lesioned 73.5±8.5 %) and in water maze test (escape latency: intact 9.4±1.5 sec, lesioned 65.5±18.1 sec). Both aFGF and bFGF significantly ameliorated the spatial memory deficits induced by F-F lesion in dose dependent manners(correct response: 400 ng of aFGF 85.4±6.0 %, 400 ng of bFGF 73.0±6.9 % escape latency: 400 ng of aFGF 11.0±1.2 sec, 400 ng of bFGF 12.8±1.2 sec). Reduction of hippocampal choline acetyltransferase activity caused by F-F lesion was not affected by the injection of aFGF and bFGF. In conclusion, we suggest that delayed administration of aFGF and bFGF improve the surgically induced spatial memory disorders without affecting the hippocampal cholinergic transmission. Further investigations would differentiate the long-term (neurotrophic) and short-term (neuromodulatory) actions of FGFS on learning and memory.
641.7 EFFECTS OF CHRONIC dDC ADMINISTRATION ON LOCOMOTOR ACTIVITY IN RATS. H.D. Davis, D.E. Morey, and N. E. Green. YDA, Antiviral Research Laboratory, Rockefeller, MD 20897. *Universities Service Center, University of the Health Sciences, Medical Psychology Department, Bethesda MD 20814.

Dideoxycytidine (ddC) has been used in the treatment of HIV infection. Unfortunately, the long-term use of ddC has been associated with an apparent peripheral neuropathy and debilitating pain in a subset of HIV patients. Recently, we reported on the value of a behavioral paradigm in rats to study the neurotoxicity of the nucleoside analog and found that locomotor activity was altered in a U-shaped dose-effect relationship following increasing doses of dDC (50) to rats. The present experiment examined the effects of chronic dDC administration (PO, 0.1-10.0 mg/ml in the drinking water) on the locomotor activity of female Sprague-Dawley rats. Activity was monitored for one hour each day for eight consecutive days during a period of continuous drug administration. Body weight, food and water consumption were also monitored. Exposure to dDC at 3.0-10.0 mg/ml concentrations resulted in a significant decrement in locomotor activity beginning with day 5 of exposure. While water consumption was reduced somewhat among animals in the high drug concentration group, this occurred during days 3-5 of exposure and was not correlated with the observed locomotor decrements. Body weight, food and water consumption among all other dose groups was indistinguishable from that of the control group. Chronic dDC thus revealed a pattern of behavioral toxicity that an acute paradigm could not detect. Implications of these findings for the neurotoxicity testing of drugs used in AIDS therapy will be discussed.


The methionine and tyrosine catabolites have been shown to mitigate the locomotor deficit that follows administration of ammonium radioprotective compounds. A likely mechanism of action for this effect is theophylline's antagonism of the adenosine (A) system. This study compared the effects of nonselective adenosine agonists (theophylline) with those of selective A2 receptor agonists (8-Cyclopentyl-1,3-dimethylxanthine, CPT) and A1 (3,7-Dimethyl-1-propargylxanthine, DMP) receptor antagonists (8-Cyclopentyl-1,3-dimethylxanthine). Locomotor activity (total distance travelled) was examined in naive CDF1 rats in an automated activity monitor for 12 hr postinjection. The effects of WR-151327 alone and in combination with adenosine agonists on locomotor activity were examined. WR-151327 (200 mg/kg, i.p.) alone significantly reduced activity from 30 min to 3 hr postinjection. The adenosine antagonists (20 mg/kg, i.p.) were each administered 30 min prior to WR-151327 (200 mg/kg, i.p.) at which time locomotor activity monitoring began. Compared with vehicle controls, theophylline alone significantly increased activity for 1 hr, and caffeine alone for 2 hr. CPT alone increased activity for 0.5 hr, and DMPX alone increased activity for 2 hr. When combined with WR-151327, theophylline reversed the deficit for 1 hr, while CPT failed to mitigate the deficit. These results provide evidence that the A2 adenosine receptor is important in mitigating WR-151327-induced locomotor activity decrements.

641.9 EVALUATION OF DOSE-RELATED EFFECTS OF D-CYCLOSERINE IN UNMEDIATED OBSESSIVE COMPULSIVE DISORDER PATIENTS. Barr LC, Bennett A, D’Souza DC, Price LH, Krystal A. Yale Univ Sch of Med, New Haven, CT 06519.

Functional neuramigating studies implicate orbital frontal cortex and caudate in the mediation of obsessive compulsive symptoms. As excitatory transmission within and from frontal cortex to striatum is prominent, the effects of D-cycloserine, a partial agonist at the glycine site of the NMDA receptorpar, were evaluated in the present study. Method: 11 unmedicated Obsessive Compulsive Disorder (OCID) patients (mean ± SD age: 39±6; 12 male, 5 female) received either a placebo, a 50 or 150 mg intravenous dose of D-cycloserine on three test days each repeated for 2 weeks. A 15 point scale was used to assess obsessions and compulsions. Possible effects of D-cycloserine on frontal and memory function were assessed via various tests: Hopkins Verbal Learning Test, Nonverbal Selective Reminding Test, Rey Taylor Complex Figure Test and a test of verbal fluency. Results: D-cycloserine has been shown to increase cortical in healthy subjects, cortisol was measured at baseline and following D-cycloserine. Data were analyzed using RM-ANOVA. Results: No dose or dose x time effects were identified for clinician or patient-rated obsessions and compulsions and no clinically apparent changes in symptom severity were observed. Neuropsychological testing also did not identify medication effects. Data comparing the hormonal response of these patients with healthy subjects will be presented. Conclusions: Putative facilitation of excitatory transmission produced by D-cycloserine may not markedly effect OC symptoms or neuropsychological deficits sometimes observed in these patients.


Disulfiram is the most commonly used treatment for alcoholism. Despite its established use, the research literature is sparse regarding the central neurochemical or behavioral toxicological effects of this important medication. We recently observed (Mueller, Husten, Matha, & Elper, 1994) that disulfiram on a marked inhibition in the biosynthesis of a-aminated peptides which persists for weeks after the cessation of treatment. More than half of the peptides used in intercellular communication are a-aminated and in nearly all cases this modification is required for receptor binding and biologic activity. The present experiment was designed to evaluate the effects of disulfiram administration on behavioral measures of locomotion (hot plate and tail flick), peripheral muscle performance (grip strength), motivated performance, balance, and coordination (rotorod), body weight; and pentylylglycine-N-hydroxylating monooxygenase (PHM) activity in the hypothalamus, neuro-intermediate lobe of the pituitary (NIL), adenohypophysis, and brain; and a-MSH levels in the neurohypophysis. Adult, male, Sprague-Dawley rats were treated daily for one week with disulfiram (50, 150 mg/kg, s.c) and were evaluated for two weeks after cessation of treatment. Disulfiram significantly increased hot plate response latencies, decreased grip strength, decreased rotorod performance, and decreased body weight in a dose-response manner. These effects persisted through the two week post-treatment period and paralleled the time course of the inhibition of PHM previously reported in addition, disulfiram significantly altered PHM activity in tissue samples from the NIL, and adenohypophysis, and a significant decrease in a-MSH. These findings indicate that disulfiram may have several untoward effects that are relevant to therapeutic use and warrant further investigation.


Acute exposure to cold stress has been reported to impair performance on a group of tasks, including an increase in the speed with which behavioral and neural operations occur. Exposure to a temperature of 2°C increases rate of responding on a time-based differential-reinforcement-of-low-rate (DRL) reinforcement schedule, without affecting attentional, sensory, or motor aspects. Administration of the amino acid, L-tyrosine, has been shown to improve behavioral impairments induced by exposure to cold. This present study examined the effects of tyrosine in rats performing on a DRL schedule that were exposed to a decrease in temperature. Prior to treatment, rats were trained to respond to a DRL schedule with a 120 s time-out for each trial. Following training, rats were divided into control and cold exposure groups (50/50). Cold exposure consisted of a 60 min cold exposure session, in which the temperature was maintained at 2°C for 60 min. Following the cold exposure, rats were tested on the DRL schedule for 1 hr. Rats that received no tyrosine (50mg/kg) exhibited a decrease in the number of responses made. Rats that received tyrosine (50mg/kg) prior to cold exposure, however, showed a significant increase in the number of responses made. This increase persisted for 2 hr following cold exposure, suggesting a rather direct effect of tyrosine on cold-stress impairment of DRL performance.


Two groups (N=10) received pairings of a distinctive environment, the laboratory, and administrations of 700 mg of the muscle relaxant carisoprodol (Carisoprodol, CP) and saline. CP pairings of the same environment with administrations of an inactive agent (Control group) for a total of three sessions, each spaced one week apart. The laboratory was hypothesized to constitute a conditioned stimulus (CS) for the drug unconditioned stimulus (US) in the Carisoprodol group. In the fourth session the Carisoprodol group received the inactive agent in the laboratory, and the Control group received carisoprodol in the laboratory. Dependent variables were blink reflexes and carisoprodol serum concentration. Results showed facilitated blink reflex amplitudes in the Carisoprodol group when an inactive agent was administered in the laboratory, which is opposite to the effects of carisoprodol. This indicates that a conditioned drug antagonistic response was elicited by the CS in this group. Moreover, blink reflex amplitudes were inhibited in the Control group when it received carisoprodol, indicating that an antagonistic response was not elicited in this group.

SPOR: European Brain and Behaviour Society
641.13


In the present study, rats (N=12) were trained to discriminate the voltage-gated potassium channel blocker 4-aminopyridine (4-AP) (1.0 mg/kg, i.p. or 1.7 mg/kg, i.v., 30 minute pretreatment) from saline in a two-drug discrimination procedure. Rats responded differentially on the left and right lever under a 3A response fixed-ratio schedule of food presentation depending on whether 4-AP or saline was administered. 4-AP served as a discriminative-stimulus in all the rats, showing dose-dependent (1.7 mg/kg) increases in drug lever responses up to the training dose. Administration of saline resulted in <1% drug lever responding. There was no effect on response rates for the rats with a training dose of 1.0 mg/kg, and a moderate dose-related decrease in response rate for rats with a training dose of 1.7 mg/kg. Once the discrimination was established, the discriminative-stimulus effects of 4-AP were compared to several known potassium channel blockers, a calcium channel blocker and an acetylcholinesterase inhibitor. The structurally related potassium channel blocker, 3,4 diaminopyridine (0.3 - 17.5 mg/kg) fully substituted at doses which moderately decreased response rate in 4 out of 4 rats trained at 1.0 mg/kg. 3,4 diaminopyridine did not substitute in 2 rats that were trained at 1.7 mg/kg. Other potassium channel blockers, including quinidine (30.0 - 100.0 mg/kg) and amiodarone (30.0 - 56.0 mg/kg), a C2+ channel opener, Bay K 8644 (0.3 - 1.0 mg/kg), and an acetylcholinesterase inhibitor, 9-amino-1,2,3,4-tetrahydroacridine (THA) (1.0 - 5.6 mg/kg), generally did not substitute up to doses that decreased response rate. The present results demonstrate that 4-AP can serve as a discriminative-stimulus in rats. In view of the substitution of 3,4 diaminopyridine, but not quinidine or amiodarone, the present results suggest that the discriminative-stimulus effects of 4-AP are mediated by a subset of voltage-gated potassium channels.

641.15

NEUROPEPTIDE-Y ALTERS THE ACQUISITION OF RESPONSE SEQUENCES IN RATS. L. Scholl and J. X. Thomas. Naval Medical Research Institute, Bethesda, MD 20889-5607.

Administration of neuropeptide-Y (NPY) has been shown to both improve and impair working memory as measured with a delayed matching-to-sample (DMS) procedure. The present study used a repeated acquisition (RA) procedure to investigate the effect of NPY (2.0 to 34.0 ug/kg, iv) on the acquisition of three-member response sequences in rats. Each incorrect response in this procedure results in a brief timeout from reinforcement. Sessions were divided into a learning and a performance phase by imposing a five-minute delay at the mid point. A biphasic response, followed by performance, was observed during control sessions. The average number of error responses was 29% during the last half and 15% during the 2nd half of each session. Administration of NPY resulted in improved performance at a dose of 8.6 ug/kg and markedly disrupted performance at a dose of 34.0 ug/kg. The last half vs. 2nd half incorrect responses at the 8.6 ug/kg dose were 316, while the same measures with the 34.0 ug/kg dose were 520 and 214, respectively. NPY resulted in no effect on performance. The RA procedure contains elements of both working and reference memory. Working memory is requisite for acquiring the novel sequence of lever press each day, while reference memory is invoked by the timeout stimulus. NPY administration altered both working and reference memory aspects of the procedure. These results are consistent with and extend the finding that NPY altered working memory in a DMS procedure.

641.14

HORSE SERUM BUTYRYLCHOLINESTERASE PROTECTS AGAINST MEPO-INDUCED RESPONSE SUPPRESSION IN RATS. R. Genovesi*, R. Larrion and B.P. Doctor. Division of Neuropsychiatry and Biochemistry, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

New therapies against organophosphorus (OP) toxicity have focused on the administration of enzyme "scavengers", in order to prevent deleterious effects by neutralizing the OP before target areas are affected. We examined the ability of horse serum butyrylcholinesterase (HS-BChe) to attenuate the effects of the OP, MEPQ, in rats trained to lever press under a variable interval 56 second schedule of food reinforcement. Under baseline conditions, the schedule produced consistent and relatively fast rates of responding throughout the 60 min sessions. MEPQ (32ug/kg, i.p (n=5)) produced complete or nearly complete response suppression in rats pretreated with vehicle. Rats treated with 7500 U HS-BChe (i.p. (n=5)) approximately 1h before MEPQ injection, however, responded at nearly 70% of control rates. Blood BChe activity was elevated in rats receiving HS-BChe (34.6 U/ml) as compared to rats receiving vehicle (0.8-1.6 U/ml), but decreased when measured 1h following MEPQ administration. These results demonstrate that administration of HS-BChe can attenuate the toxic effects of MEPQ, and merits consideration as a prophylactic therapy against OP exposure. These results also confirm and extend previous reports demonstrating that protection against OP toxicity can be conferred by a number of cholinesterases.

642.1

PERSISTENT ELEVATION OF VASOPRESSIN mRNA AFTER SALT-LOADING. M.D. Fitzsimmons*, M.M. Roberts, A.G. Robinson Department of Endocrinology, University of Pittsburgh, PA 15261

Based on computer modeling, we have suggested that pituitary vasopressin (AVP) levels depleted after chronic salt-loading recover to age-matched control levels simply by translating mRNA that accumulates during the period of salt-refeeding (AmPhysiol 262: R1121, Endocrinology 134:1874). The amount of mRNA needed for such recovery depends on the threshold for stimulation. But the smallest reasonable estimate would require a 4-fold increase over baseline. Experimental measurements in our lab with RNAase protection assay of whole hypothalamus show that increasing salt-loading increases AVP mRNA expression by only 1.5-2.5 times, a range consistent with other recent reports. These results might be interpreted to indicate that translational regulation plays a role in the regulated expression of pituitary vasopressin levels. Alternatively, mRNA may remain elevated after the stimulus is removed. Evidence from Zieg et al (J BiolChem 261:12956) and Arnaud et al (Neurocell Letters 149:177) supports the latter. We tested the impact of stimulatory duration on vasopressin mRNA levels after salt-loading. Both 3 days and 5 days of salt-loading result in a significant increase in mRNA vs. control (1.6-fold and 2.1-fold significantly greater, p<0.001), with 5 days being significantly greater (p<0.01). AVP mRNA showed no detectable decrease after two days of recovery in both the day 3 and the 5 day groups; the 3 day group remains elevated even 4 days after recovery (p<0.01). Plasma sodium levels in the salt-loaded groups increased significantly (p<0.001), but were not different from baseline in the recovered groups. These results demonstrate that the length of salt-loading affects the magnitude of AVP mRNA increases and that the post-stimulus elevation of mRNA is independent of stimulus duration. Whether the persistence of AVP mRNA is due to "membrane" in the transcriptional regulation of the gene or to increased mRNA stability will be determined with in vitro hybridization. In either case, the unexpectedly prolonged mRNA elevation probably contributes to the recovery of pituitary vasopressin content to age-matched levels after salt-loading.

642.2


Sustained hyposomorality enhances the abundance of OT and AVP mRNAs in the rat hypothalamus. Gonadal steroids modify this response. Gonadectomy prevents, and gonadal steroids restores, OT and AVP expression to osmotically-challenged male. OT gene is influenced by other hormones, including thyroid hormone(T4). We questioned whether T4 deficiency may also alter OT and AVP mRNA abundance in the paraventricular nucleus (PVN) of the osmotically-challenged rat. Male Sprague-Dawley rats (7 wks of age) were sham thyroidectomized (sham TX) or TX, given T4 on an osmotically-challenging diet, and then later with empty (TX+sham) or T4-added (TX+T4) capsules, randomized to 2% NaCl solution or tap H2O for 5-10 days and sacrificed at the end of the NaCl challenge. Serum T4 levels were significantly lower in the TX vs. sham TX and TX-T4 groups (p<.0001). All NaCl groups developed hyperammonia and depleted pituitary stores of AVP. Northern blot hybridization showed that salt loading increased PVN OT and AVP mRNAs in all groups compared to their respective tap H2O controls. In contrast to the effect of gonadectomy, hypothyroidism does not alter osmotically-stimulated-accumulation of OT and AVP mRNAs in the rat PVN.
643.3

BCI RNA IN THE RAT POSTERIOR PITUITARY: AXONAL LOCALIZATION AND REGULATION UPON DEHYDRATION AND REHYDRATION. A. Zhrebchev* and F. E. Bloom. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Brain cytoplast (BCI) RNA, a small non-translation RNA-polymerease III transcript, has recently been found in the posterior pituitary (PP) using a low resolution radioactive in situ hybridization (ISH) approach, and it has been hypothesized that this transcription might be autoregulated (Snedeker et al., Cell, 1989; Tisdale et al., J. Neurosci., 13:4214-4219, 1993). In the present study, we aimed to determine whether BCI was truly located within axons, rather than in the glial cells of the PP, and further to examine the possible regulation of this RNA upon dehydration (chronic salt-loading, providing 2% of NaCl as exclusive source of water for 3, 5, and 7 days) and rehydration (return to regular water beginning after day 7 of salt-loading, for 1, 2, 3, or 4 days). A BCI Northern blot assay, we were able to detect BCI RNA in both the anterior and neurointermediate lobes of the pituitary. Non-radioactive ISH of BCI RNA performed on PP at both the light and electron microscopic levels revealed that BCI RNA was axonally compartmentalized. Using a semi-quantitative ISH approach, we have measured and compared the changes in BCI RNA and vasopressin (VP) mRNA during dehydration and rehydration. During dehydration, BCI RNA was significantly increased, but it already reached a maximum at day 3 of salt-loading, while VP mRNA was more progressively increased during the full period of salt-loading, peaking at day 7. The increase in BCI RNA labeling (2-5 fold) was however modest compared to the increase in VP mRNA labeling (around 1-11 fold). Upon the rehydration, the BCI RNA content in the PP was reversed to control value as early as 1 day after the onset of rehydration, while VP mRNA slowly decreased to reach the control value at day 7. In conclusion, like VP mRNA, BCI RNA is transported in axons of the hypothalmo-neurohypophysial system, and its axonal transport is similarly regulated. Therefore, we propose that BCI RNA might be involved in either the axonal targeting and/or transport of VP and other axonal mRNAs.

645.5

Testosterone (T) Enhances Vasopressin Messenger Ribonucleic Acid (AVP messenger) Abundance in the Hypothalamus of Osmosomatically-Stimulated Female Rats. J.A. D'Kraeke*, N.B. Kim, and A. Amico. Dept of Medicine, University of Pittsburgh School of Medicine and VA Medical Center, Pittsburgh, PA 15261.

Gonadectomy (gdx) prevents enhanced accumulation of AVP mRNA in the hypothalamic paraventricular nucleus (SON) of osmotically-challenged male and female rats and T and dihydrotestosterone (DHT) restore this response in the male rat (Soc. N.S. Abs. 530:2:1280, 1993). We questioned whether AVP mRNA in the SON of osmotically-stimulated rats display a sexually dimorphic response to gonadal steroid replacement. We examined the effects of various gonadal steroid replacement regimens upon AVP mRNA accumulation in the SON of osmotically-stimulated female rats. Female rats were gdx or sham gdx (intact) and implanted with sham, T or estradiol (E2) capsules and randomized to either 2% NaCl solution or tap H2O x 5 days. NaCl groups lost weight, developed hypernatremia and depleased their pituitary stores of AVP compared to tap H2O groups (p<0.05, Fisher's LSD). Gdx-T and gdx-E2 treated animals had mean T and E2 concentrations of 1.5 ng/ml and 60 pg/ml, respectively. Intact and gdsx-T-replaced rats, but not gdx or gdx-E2, rats receiving 2% NaCl increased hypothalamic AVP mRNA accumulation compared to respective tap H2O controls (p<0.05, Scheffe F-test). The data suggest that similar to male rats, T, but not E2, modulates AVP mRNA accumulation in the SON of osmotically-challenged female rats.

645.6


The distribution of AVT containing neurons in quail brain has been studied in detail by immunocytochemistry (ICC). However no data are available for this species on the gene level. Up to now the chicken represents the only avian species in which AVT gene expression has been examined using cDNA probes. Thus we have used a gene probe directed to the 5' part of the AVT mRNA in order to detect the distributions of AVT expressing neurons in chicken and quail brain. Northern and Southern analysis demonstrated the specificity of this probe for quail AVT mRNA. 32nds thick cyrosection from chicken and quail brain were collected on different slides then adjacent slices. Consecutive were fixed by 4% paraformaldehyde and processed for in situ hybridization (ISH) and ICC respectively. The results indicate a common pattern in the distribution of AVT neurons containing mRNA and the peptide. In particular a wide distribution of perikarya was observed close to the pial surface of the preoptic region (lateral or supraoptic division), in the periventricular hypothalamic region (paraventricular nucleus) and scattered along the lateral forebrain bundle. Few neurons were observed by the ICC in circumventricular areas. Quail and chicken AVT mRNA expressing neurons showed a common pattern of distribution, confirming the suitability of the probe. Considerably more AVT expressing neurons were detected in the hypothalamus of the chicken if compared to the quail. Furthermore the hybridisation signal was more intense in the brain of chicken than in quail. In conclusion our studies show that 1) the probe we used is suitable to investigate AVT gene expression in quail, 2) the localization of AVT neurons is comparable between chicken and quail, 3) the basal activity of the AVT system in chicken is higher than that in quail. Supported by grants of CNR. N.A. was granted by a ENP short term fellowship.

645.8

AP-1 DNA BINDING ACTIVITY INDUCED BY HYPEROSMOLALITY IN THE RAT HYPOTHALAMIC SUPRAOPTIC AND PARAVENTRICULAR NUCLEI. C. Ying*, D. Reisman, and J. Buggsy. Deps. of Physiology and Biological Science, University of South Carolina, Columbia, SC 29208.

The immediate early gene (IEG) c-fos is induced upon hyperosmotic stimulation in the paraventricular (PVN) and supraoptic (SON) hypothalamic magnocellular nuclei. AP-1 DNA binding activity was examined in these brain regions since Fos acts as a transcription factor in other systems by binding to the AP-1 site on DNA to influence gene transcription. Rats were given hypertonic saline injection (i.p.) and nuclear proteins were extracted from PVN and SON tissue punches 2 hrs later to assess AP-1 binding activity using an electrophoretic mobility-shift assay and a labeled oligonucleotide containing the AP-1 consensus sequence. Hyperosmolality induced AP-1 DNA binding in both PVN and SON; this binding was competitively displaced by excess unlabeled IEG. The binding of proteins to the AP-1 element was specific to AP-1 consensus binding sites utilizing competitor DNAs such as SP-1. Thus, AP-1 binding activity in nuclear extracts from the SON and PVN was induced after hyperosmolality suggesting that dehydration-induced IEG expression results in protein products that may function in the regulation of target gene expression.
**462.9**

EVIDENCE FOR A FUNCTIONAL PROJECTION TO THE LATERAL HYPOTHALAMUS FROM THE MEDIAN PREOPTIC NUCLEUS AND SUBHYPOTHALAMIC ORGAN IN THE RAT. A.B. Kelly* & A.G. Wang. NIH Program and Department of Biological Sciences, UIC, Los Angeles, CA 90086-2200.

We have previously reported increased levels of CRH mRNA in the lateral hypothalamic area (LHA) in response to cellular dehydration caused by increased plasma osmolality. We have now combined tracer and hybridization in an attempt to identify those neurons responsible for this response. Following isotopomers of fluorescent (FG) into the LHA we identified retrogradely labeled somata of a number of cell groups, including the subfornical organ (SFO), median preoptic nucleus (MP0), bed nucleus of the stria terminalis, preoptic area, and other forebrain regions. We then combined FG retrograde tracing from the LHA with a c-fos antiserum that detects the expression of the c-fos proto-oncogene in hyperosmotic conditions. This interaction of FG-labeled cells with c-fos mRNA in LHA revealed hybridization in an attempt to identify those neurons responsible for this response. Following isotopomers of fluorescent (FG) into the LHA we identified retrogradely labeled somata of a number of cell groups, including the subfornical organ (SFO), median preoptic nucleus (MP0), bed nucleus of the stria terminalis, preoptic area, and other forebrain regions. We then combined FG retrograde tracing from the LHA with a c-fos antiserum that detects the expression of the c-fos proto-oncogene in hyperosmotic conditions. This interaction of FG-labeled cells with c-fos mRNA in LHA revealed hybridization in an attempt to identify those neurons responsible for this response.

**462.10**

SEPTAL VASOPRESSIN AND OSMOTIC REGULATION. I. Zhang*, C. Hopkins*, G. de Yates. Dept. of Psychology, Prog. in Neurosci. & Behav., Univ. of Massachusetts, Amherst, MA 01003; & Dept. of Psychology, Smith College, Northampton, MA 01060.

Sepal areas have been found to play an important role in the regulation of water intake and vasopressin (AVP) neurosecretion. Several studies have suggested that the AVP insertion of the septal may play a role in this regulation. In dogs, intraventricular injections of AVP lowered the hyperosmotic thirst threshold whereas injections of AVP antagonist raised it. In rats, hyperosmotic and hypovolemic stimuli decreased AVP content in the lateral septum, and increased AVP release from this area. AVP-immunoreactive fibers in the lateral septum (LS) arise from the bed nucleus of the stria terminalis and medial amygdaloid nucleus. AVP expression in these nuclei depends on the presence of gonadal steroids. If the AVP innervation of the lateral septum is indeed involved in water intake, one would predict that this function is influenced by gonadal steroids. Here we showed that this is the case. We found that when challenged with the same amount of hypertonic saline, injected intraperitoneally, castrated male rats drank less than intact rats and castrated rats treated with testosterone did. AVP injections into the lateral septum of castrated rats partly restored the amount of water intake. AVP injections into the lateral septum of intact rats increased, and AVP antagonist injections decreased, water intake in response to hypertonic saline. We also found that hypertonic saline injections increased c-fos and egr-1 expression in lateral septum neurons as well as in neurosecretory AVP neurons in the paraventricular and supraoptic nucleus.

This increase was more pronounced in intact than in castrated rats suggesting that the septum may also mediate steroid effects on AVP neurosecretion.

**462.11**


Fos and Jun, protein products of the immediate early genes c-fos and c-jun, form a heterodimeric complex that affects the transcription of genes containing AP-1 and CRE DNA-binding sites. Using a double-label immunofluorescence method (C&L & Tissue Research, 1994, 276:1-6), we detected Fos/Jun immunoreactivity in the hypothalamic nuclei in the appearance Fos and Jun in the nuclei of SON neurons. Cell counts of immunostained sections demonstrated the co-exist and appearance of Fos and Jun occurred within 30 min (20%), peaked at 90-120 min (80%) and gradually disappeared by 4 hr (13%) after injection. At 4 hr post injection, 5 rats each received a second injection of normal or hypertonic saline. A second injection of normal saline resulted in no Fos/Jun immunostaining 90 min later, while hypertonic saline induced Fos/Jun staining in only 17% of SON neurons. Of the remaining SON cells, 23% had staining to Fos alone and 4% of the cells stained for Jun. These results indicate SON neurons, after a second hypertonic saline injection, exhibit decreased colocalized Fos/Jun immunostaining, dramatically decreased Jun expression, and substantial, but attenuated, immunostaining for Fos. Supported by grants NIH NS25913 and USUHS RO1S to JT.

**462.12**


This study was to evaluate adaptation of the brain to hyponatremia in a gender of function. Brain adaptation to vasopressin (AVP)-induced hyponatremia was studied in adult male and female cats and rabbits during 3 hours or 4 days of hyponatremia, which was induced by subcutaneous AVP and 140 mM glucose/H2O. Changes in cerebral blood flow were evaluated using contrast- enhanced high speed echo-planar MRI, using bolus IV administration of a magnetic susceptibility contrast agent (Gd(DTPA)-BMA). An index of cerebral perfusion (CPI) was obtained as a reciprocal of the width of the contrast transverse. Water, sodium and potassium content were assessed in brain gray matter of both cats and rabbits. Acute hyponatremia (2-4 h; serum sodium fell from 155±3 to 127±3 mmol/L) resulted in a significant prolongation of contrast transit time through the brains of both male and female cats: CPI decreased from 0.24±0·02 sec−1 to 0.14±0·02 sec−1 in males and 0.15±0·02 sec−1 in females. Adaptation was gender dependent: brain (gray matter) water content increased from 391±8% to 434±5% in females and 415±3% in males (p=0.001 vs control), and hyponeptic females had greater than males (p=0·02). A gender dependent decrease of brain sodium was found, from 271±13 mmol/kg dry tissue to 222±2 in males (p=0·005) and to 233±5 in females (p=0·035). In rabbits, serum sodium was decreased from 142±1 to 122±2 mmol/L over 4 days. Brain water increased from 423±13% to 492±27% in females (p<0·01) and 432±27% in males (p<0·01). Brain sodium fell from 318±6 to 294±8 mmol/kg dry wt in males (p<0·01) but was unaffected in females. Brain potassium did not change. Brain swelling, however, was significantly less in hyponeptic males than in females in both species. Better adaptation of brain to hyponatremia in males of 2 species, and decreased cerebral perfusion, appears to be related to interaction between AVP and sex steroid hormones.

**462.13**

VENTRAL FOREBRAIN RELAYS SALT INPUT FROM GUT TO VASOPRESSIN NEURONS. Y. Shin, M. King and A.J. Gaertner*. Physiology Dept., Univ. of Virginia, Charlottesville, VA 22908, and Stetson College, FLA.

Gastric hypertonic saline infusions (600 mg/kg, 2.5mI) elicit vasopressin (AVP) release in conscious rats via neuroregulatory pathways ascending from Rf/R2 (Brain Res, 380:181-91,1992). Microinjections of 5DHA (4µg/400nl) into magnocellular hypothalamic nucleus, caudal DBH. The 5DHA lesions were from 0.5 mm to 2.02±0·45 mg/ml (p<0·01). Electrolyte lesions in the hypothalamic nuclei decreased mean AVP from 2.02±0·45 mg/ml (p=0·07). Electrolyte lesions in the hypothalamic nuclei decreased mean AVP from 2.02±0.45 mg/ml (p<0.01). These results suggest that salt signaling by the gut is mediated by neuroregulatory mechanisms ascending probably via MFB to ventral forebrain, and via non-catecholaminergic fibres descending to the vasopressinergic nuclei (support: NIH N27644).

**462.14**


Neurons within the OVLT and MnPo have been implicated to play a key role in osmoregulation. To determine whether they are intrinsically sensitive to hypertonicity, patch clamp recordings were obtained from cells acutely dissociated from these regions as described by Ollot and Bourque (J. Physiol. 445, 1992). Neurons were plated on petri dishes and superfused with a HEPES-buffered medium. Recordings were obtained from phase-bright somata with mean cross-sectional diameters ranging from 22 µm. Hypertonic stimuli (+10 to +30 mM Mannitol) caused a reversible and reproducible increase in the mean firing rate in 2 of 5 MNp and 9 of 14 OVLT cells tested. These effects were associated with a membrane depolarization of 32 mV and decrease in input resistance (n=14). Analysis of voltage-current relationships obtained before and during the osmotically-evoked depolarization revealed a reversal potential near -45 mV. Furthermore, the EcL was -108 mV, suggesting the possible involvement of calcium currents. These results demonstrate that some neurons within the OVLT and MnPo are intrinsically sensitive to hypertonicity. Supported by the MRC and the Heart & Stroke Foundation of Canada.

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642.15

The release of neurohypophysial hormones from supraoptic neuron terminals is regulated by plasma osmolality. Recent biophysical studies in our laboratory have suggested a role for mechanosensitive cationic channels in the sensitivity of supraoptic neurons to changes in external osmotic pressure. The involvement of such channels in osmoreception was assessed by the use of gadolinium (Gd^3+), a divalent cation known to block different types of stretch-sensitive cationic channels. Whole-cell recordings obtained from isolated rat supraoptic neurons revealed a marked reduction of hyperosmotically-mediated membrane depolarization and firing rate increase in presence of Gd^3+ (100µM, n=4). In voltage-clamp mode (I=10), bath-applied Gd^3+ induced a reversible and dose-dependent inhibition (IC50=20µM) of the inward current elicited at -60mV by hypertonic stimulation. This inhibition resulted from specific suppression of the cationic conductance which was previously shown to be associated with osmotic responses (E<sub>H</sub>=+40mV). In cell-attached experiments (n=15), Gd^3+ was also able to block mechanosensitive cationic channels when included or perfused in the recording pipette. These results confirm a role for mechanically-gated channels in the endogenous osmosensitivity of rat supraoptic neurons.

Supported by the MHC and Heart & Stroke Foundation of Canada.

642.17
ENHANCED VASOPRESSIN AND BEHAVIORAL RESPONSE TO METHYLPHENIDATE IN SCHIZOPHRENIC'S WITH WATER INTOXICATION M.B. Goldman, D.J. Lachina and G.L. Robertson Illinois State Psychiatric Institute, University of Chicago Pritzker School of Medicine, Chicago IL, 64675.

Water intoxication accounts for about 20% of deaths in non-geriatric schizophrenics. This episodic disorder is associated with intraseat impairments in water excretion which occur during exacerbations of psychosis. Many other schizophrenics remain normonatremic during such exacerbations, even if they are polydipsic. We administered the psychomimetic, methylphenidate (0.5 mg/kg intravenously) to concurrent schizophrenics with and without a previous history of water intoxication, in order to further examine the relationship of psychosis to water imbalance.

Baseline AVP was maximally suppressed in both groups, but peak post-methylphenidate levels were greater in time - 15 min. (M±SD) 1.9 ± 0.7 pg/ml vs control (1.0 ± 0.7, t = 2.21 df = 12 p < 0.05) subjects, despite lower plasma osmolality in the former (T = 278 ± 1.5 mosm/Kg, C = 288 ± 1.5). AVP stimuli (e.g. nausea, abdominal discomfort, hypotension) were not present in any subjects, and parameters influencing AVP release were similar in both groups over the two hours post-methylphenidate. Desire for water was also similar and did not change. Positive symptoms of psychoses increased, and negative symptoms diminished, equivalently in the two groups. Test subjects, however, were more psychotic and showed a greater increase in other psychotic symptoms.

Methylphenidate causes greater AVP secretion and behavioral activation in schizophrenics with episodic water intoxication. These findings strengthen the link between psychotic exacerbations and vasopressin secretion, and suggest the former is not necessarily a consequence of hyponatremia, per se. Differences in limbic pathology between schizophrenics could account for the findings.

SOMATIC AND VISCERAL AFFERENTS: NOCICEPTION

643.1

In contrast to myelin-forming Schwann cells, little is known about the factors regulating Schwann cells that do not elaborate myelin sheaths. These non-myelin-forming Schwann cells co-express several surface proteins and intermediate filaments, including glial fibrillary acidic protein (GFAP), not found in myelin-forming Schwann cells. These non-myelin-forming Schwann cells are physiologically important in that they ensheath the peptide-containing sensory C-fibers, and thus may indirectly regulate the transmission of nociceptive information and the inflammatory response in which unmyelinated C-fibers are involved. In the present study we have used combined receptor autoradiography and homogenate receptor binding methods to demonstrate that the same non-myelin-forming Schwann cells that are GFAP+ also express high concentrations of [125I]Endothelin receptor binding sites. Pharmacological analysis suggests that these binding sites correspond to Endothelin-B receptors. These results suggest a mechanism by which a circulating factor such as endothelin may specifically, yet indirectly, affect the population of non-myelinated primary afferents that are hyperalgesic to be involved in transmitting chronic pain and in regulating the inflammatory response in peripheral tissues. (Supported by the NIH and VA).

643.2

Noradrenaline has been shown to modulate some trigeminal and dorsal root ganglion (DRG) neurons, but the nature of noradrenaline actions and the adrenergic receptor subtype(s) mediating these effects have not been explored fully. Quantitative receptor autoradiography and homogenate receptor binding was used to locate and characterize the adrenergic receptor binding sites expressed by the cells comprising the trigeminal and dorsal root ganglia in the rabbit, rabbit, and monkey. [3H]-HEAT was used to label the a<sub>1</sub> binding sites, [3H]-Iodo-cocclodine was used to label the a<sub>2</sub> binding sites, and [3H]-cyanopindolol was used to label the b, and b<sub>2</sub> binding sites. In the rat, rabbit, and monkey DRGs a high concentration of b<sub>2</sub> adrenergic receptor binding sites were observed, with moderate concentrations of a<sub>1</sub> binding sites and low but detectable concentrations of a<sub>2</sub> and b<sub>1</sub> receptor binding sites. Within the DRG, specific binding sites corresponding to all 4 subtypes of adrenergic receptors were associated with neurons and the supporting cells, fibroblasts or endothelial cells. That all 4 adrenergic receptors are expressed primarily by neurons is supported by the observation that after double ligation of the sciatic nerve, build up of adrenergic receptors and the affinity of these adrenergic receptors are appropriately positioned to presynaptically modulate primary afferent neurons. (Supported by the NIH and VA).
Role of a Slow Ca\textsuperscript{2+}-dependent Afterhyperpolarization in Protaglandin E\textsubscript{2}–Induced Sensitization of Cultured Rat Sensory Neurons. Michael S. Gold\textsuperscript{a}, Michael J. Shuster, Shahrab Dastmalchi\textsuperscript{a} and Jon D. Levine\textsuperscript{b}, Departments of Medicine and Oral Surgery, and Division of Neurosurgery, UCSP, Box 05745, San Francisco, CA 94141

To determine if inhibition of a slow (<1 sec) Ca\textsuperscript{2+}–dependent afterhyperpolarization (sAHP) in Protaglandin E\textsubscript{2} (PGE\textsubscript{2})–induced sensitization of dorsal root ganglion (DRG) neurons, we have used patch-clamp electrophysiological techniques on cultured DRG neurons from calves for a role of the sAHP in sensitization of DRG neurons, we demonstrate that: 1) sAHP expression is reduced to 30% of control in these neurons (21% of all cells studied) which also express properties associated with nociceptors, including small cell body size, a shoulder on the falling phase of the action potential (AP), and a rapid depolarization in response to capaci

SOMATIC

7.5) 7%.

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The results show the plasticity of isolated somata after nerve injury. Supported by NSF grant 8903577.

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56.8

PHOSPHOLIPASE A\textsubscript{2}, INDUCED ELECTROPHYSIOLOGICAL CHANGES IN RABBIT LUMBAR FACET JOINT CAPSULA. DC QUANTZ, F KALLBERG, S VAIDYANATHAN, OR LI, DC BLACQUEY, JR CAYANNAK, AT KING, Bioengineering Center, Wayne State University, Detroit, MI 48202.

The aim of this study was to investigate the histological and electrophysiological effects of phospholipase A\textsubscript{2} (PLA\textsubscript{2}) injected in the lumbar facet joint capsule. In rats, the rabbit facet joint capsule was mechanically searched for receptive fields. The units were characterized by spontaneous discharges and mechanical thresholds. 750 or 1500 U of PLA\textsubscript{2} (i.e., 15% or 50% of the buffer) were injected into the receptive field. The threshold and unit activity were recorded for later computer analysis. At the end of the experiment, the animal was histologically examined with H&E staining. REDUCTIONS 1500 U PLA\textsubscript{2}, injections (n=4): a) At 30 min, a significant decrease in amplitude (30%) was observed; b) Group II units (n=3); SDR was significantly decreased and all units disappeared between 40 and 45 min. If the SDR reached a discharge rate of 20/5 and disappeared at 7 min. 750 U PLA\textsubscript{2}, injections (n=4): a) Multi-unit SDR was decreased at 30 min. After 20 min SDR did not increase in response to 100 μl of saline (n=4) did not show any significant changes. There were also no histological changes observed in any of the tissue injections. Supported by NIH Grant RR-01933.

Substantial changes can be observed in A-delta mechanical nociceptors following injection of carrageenan (Cooper et al., 1991). This includes changes in both reactive range and mean discharge rate. It is unclear whether proinflammatory (PI) mediators make distinct contributions to sensitization. These studies examined the qualitative and temporal features of sensitization that follow bradykinin (BK), serotonin (5HT), and prostaglandins (PGE1, or PGE2). Using a force-servo stimulator, nociceptor reactivity was assessed at 5, 30, and 60 minute intervals after injection of PI agents.

Significant decreases in discharge range were observed following BK or 5HT. Changes in range were complementary, with BK producing effects within the first 5 minutes while significant effects of 5HT were delayed to the 30 and 60 minute test intervals. Neither BK or 5HT produced changes in mean discharge rate. In contrast, injections of PGE's produced delayed changes in rate of discharge (60 min) but no significant changes in reactive range. It was concluded that BK, 5HT, and PGE's made distinct and temporary contributions to mechanical sensitization. Supported by NIH DE8701.

AMPA AND NMDA RECEPTOR-IMMUNEACTIVITY POSTSYNAPTIC TO PRIMARY AFFERENT TERMINALS IN THE SUPERFICIAL CAT SPINAL CORD. E.I. Alveros*, D. Harrington and R.E.W. Price, Dept of Anatomy, Wright State University, Dayton, OH, 45435.

Primary afferent terminals believed to use excitatory amino acids (EAA) as their principal "fast" neurotransmitters. There is potential for great functional variability at EAA-synapses because of the diversity of EAA-receptor subtypes that can be expressed, details of subunit diversity are largely unknown for individual synapses. A promising approach is to use antibodies (Abs) against different subunits to probe the selectivity of postsynaptic responses using electron microscopy immunocytochemical techniques. Here, we used Abs directed against epitopes found in the GLUR2 and 3 subunits of the AMPA receptor (Chemicon) and the NR1A subunit of the NMDA receptor (Pharmacia) to detect these subunits in the superficial laminae of the cat spinal cord. The Abs labeled many postsynaptic sites and also the cytoplasm of cell somas and dendrites. All three kinds of known central glomerular types (presumed primary afferent origin) were associated with postsynaptic immunoreactivity (ir) to GLUR2/3 and NMDAR1. Interestingly, within a single glomerular terminal less than half of the synapses were postsynaptic to the postsynaptic profiles displayed cytoplasmic ir. Our results provide immunocytochemical proof supporting the assertion that most classes of primary afferents arborizing throughout laminae I to III use AMPA and NMDA receptors to elicit postsynaptic effects, but intriguingly the Abs did not universally label all primary afferent synapses despite our efforts to increase detection sensitivity to a maximum. It remains to be determined if these results are due to the presence of different subunit types, subtype isoforms, variable conformations, or posttranslational modifications modifying a primary afferent terminal.


Hyperalgesia to mechanical but not heat stimuli occurs in the uninjured skin surrounding the glabrous head (Raja et al., Brain Res. 1984). In the present study, we investigated whether secondary hyperalgesia is produced by capsaicin injected into the hairy skin. Heat and mechanical testing was done twice before, and 20 and 60 min after an intradermal injection of capsaicin (50g in 10µl) to the volar forearm of 12 volunteers. A 9 bar Von Frey filament was used to map the zone of hyperalgesia to punctate mechanical stimuli after the capsaicin injection. Our laser thermal stimulator was used for heat testing at multiple sites inside and outside the mechanical hyperalgesic zone plus the site of capsaicin injection. Subjects pressed a key to indicate heat pain threshold to a ramped (1°C/sec) heat stimulus (39 to 46°C). Subjects used a visual analog scale to rate pain to intense heat (44, 46 & 48°C, 1 or 2 duration). Sixty minutes after the capsaicin injection, pronounced secondary hyperalgesia to mechanical stimuli was present, but heat pain threshold was significantly higher (P < 0.01) and suprathreshold ratings were significantly lower (30 to 9) at the capsaicin injection site. Similar results were obtained in 5 other subjects tested with contact heat. These results suggest that secondary hyperalgesia is produced by hyperalgesia to mechanical but not to heat stimuli. Supported by NIH (NS-14447).

INTERLEUKIN-2 AND CYTOSOMATIC C POLYMOLAL NOCICEPTORS: DOSE-RESPONSE RELATION, TACHYPHYLAXY AND LACK OF SYNERGY WITH PROSTAGLANDIN F1A. I. Marini-Di Pasquale, Division of Neurology, Section of Neurosurgery, Medical School, The University of Newcastle upon Tyne, NE1 4HH, U.K.

In a previous study, we have shown that interleukin-2 (IL-2; 0.06 U/ml) activates both a third of cutaneous C-polymodal nociceptors and, more rarely, other classes of nociceptors. In the present study, we have investigated whether (i) the percentage and the response characteristics (i.e., latency, intensity, duration and pattern of discharge) of activated units are dose dependent, (ii) PGF induces a sensitization to IL-2.

Activity of single nociceptors was recorded in vivo with saphenous nerve preparations, in sedated anesthetized rats.

(i) Increasing doses of IL-2 (2, 4, 10, 20, 40 U/ml) were injected in the receptive field of 28 C-polymodal nociceptors and 6 mechanonociceptors (4 C and 2 Aa). Only 8 polymodal nociceptors were activated (28.5%). This activation appeared at 4.3 U/ml and became stronger with higher doses.

(ii) 14 C-polymodal nociceptors and 5 C-mechanoniccceptors were initially treated with PGE1 (100 ng/ml). Only 4 polymodal (28.5%) were activated by IL-2 (0.06 U/ml) and their response characteristics were unchanged, compared to control. Also, tachyphylaxis to subsequent injections of IL-2 (0.06 U/ml was unaffected by PI).

In conclusion, pruritus produced by high doses of IL-2 in the treatment of advanced cancer may result from the selective activation of a fraction of C-polymodal nociceptors, chemosensitive to this cytokine. The failure for PGF1 to enhance the chemosensitivity to IL-2 may explain the inefficiency of steroids in the treatment of such pruritus.

Most C-fiber nociceptors are polymodal and are excited by mechanical, heat and chemical stimuli. Few studies, however, have quantitatively examined responses of these nociceptors to noxious cold. It has been reported, using stimuli above 0°C, that a relatively small proportion of nociceptors are excited by noxious cold. We have reported that all Aδ nociceptors in rats are excited by noxious cold and most had response thresholds below 0°C. In the present study, cold stimuli were used to characterize responses of C-fiber nociceptors to a wide range of stimulus temperatures. Electrophysiological recordings were made from the sphenopalatine ganglion of anesthetized rats. Nociceptors were searched for by mildly pinching the skin. Once a nociceptor was identified and its receptive field mapped, conduction velocity, mechanical threshold and responses to thermal stimuli were determined. A total of 12 C nociceptors were studied. Mechanical thresholds (via Frey monofilaments) ranged from 0.63-6.3 ml. 8 of 12 nociceptors were excited by noxious heat and had a mean threshold of 44.3° ± 1.7°C. All nociceptors were excited by noxious cold. The mean response threshold was 2.8° ± 4.01°C. and thresholds ranged from 30° to 14°C. Approximately 50% of the nociceptors studied (5 of 12) had response thresholds at or above 0°C, while 7 of 12 were excited with stimulus temperatures below 0°C. Responses evoked by cold stimuli usually increased with stimulus intensity (lower stimulus temperatures).

It is concluded that unlike Ad "mechanonociceptors", C-fiber "mechanonociceptors" are excited by noxious cold stimuli. Responses of Ad and C nociceptors to cold differ in that a greater proportion of C nociceptors are excited by stimulus temperatures above 0°C (approximately 50% of C nociceptors; 10% of Ad nociceptors). Supported by NIH grants NS29567 and NS31223.

463.17

TEMPORAL CHARACTERISTICS OF ORAL TRIGEMINAL ACID SENSITIVITY. Bruce P. Bray* and Paul A. Moore, Monell Chemical Senses Center, 1500 Market St., Philadelphia, PA 19104.

Single unit studies of oral trigeminal afferents have been conducted to ascertain what types of trigeminal neurons are likely to contribute to acid irritation and pain. Acid sensitive neurons were found that were solely sensitive to acid although most also responded to cooling. Conduction velocities of acid sensitive neurons were found to be distributed bimodally, corresponding to C- and A-delta fiber types, which functionally corresponded to polymodal, and cold/cool acid sensitive neurons. Responses of acid sensitive neurons could be classified into three basic types 1) simple excitation, followed by rapid adaptation, 2) excitation followed by adaptation to sub-maximal activity, and 3) non-adapting excitatory responses of long onset latency (5-10 sec). The three types of responses corresponded to thermal thresholds (response type #1 and 2) and acid only (response type #3) type neurons. To examine whether acid stimulation exhibited the same phenomena of sensitization and de-sensitization as subcutaneous, neural recordings were obtained from fibers during repetitive lingual exposure to 150 mM pantoyc acid. Responses to both acid and cool/cold stimuli were suppressed in most classes of neurons that were initially sensitive to acid. Acid sensitization was classified into a previously cold activated sensitive acid neuron and acid sensitivity was reversibly induced in 2 previously silent neurons. Earlier studies of plasma extravasation implicated vascular processes in capsaicin-induced suppression of responses to acid. Under the same conditions that produced adaptation of responses to acid, no evidence of plasma extravasation was observed. Multiple lines of evidence suggest that acid stimuli cause modulation of sensitivity directly at transduction processes and by conduction block. The sustained noxious sensations of acidic stimuli may result from non-adapting acid sensitive neurons as well as by input from relatively fast adapting acid sensitive neurons that is sustained by central or spinal processes.

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Activity-dependent changes in fiber excitability and conduction velocity are adaptations following both natural or electrical stimulation of myelinated axons of peripheral and central nervous system. Activity-dependent changes in excitability are strong and long-lasting and have been implicated in the encoding of discharge trains, and modulation of impulse conduction at regions of low conduction safety factor. C-fibers are the most numerous axons in the sciatic nerve, and are highly heterogeneous, biochemically as well as physiologically. Previously, we have demonstrated different profiles of the activity-dependent threshold changes in sciatic C-fibers (Shin & Haywood, 1986). In this study, we have recorded the conduction velocity (CV) and subsequent conduction block were characterized following impulse activity (0.3, 1, 5, 10, 25 or 50 Hz, 1 min) in simple C-fibers (n=7, range of resting CVs: 0.25-1.72 m/sec). At 0.3 Hz, CV fell to 0.12-0.38 m/sec. At 50 Hz, CV fell to 0.73-0.08 m/sec. The same resting conduction velocities (CVs) were used to follow impulse activity (0.3, 1, 5, 10, 25 Hz, 0.01, 0.05, 0.1 Hz). The frequency of conduction block was increased as CV increased, and the activity rate (rose to 0.30, 0.15, 0.05 Hz, 25 Hz, 5.38-0.69 Hz) and most of the fibers were blocked at 50 Hz (50%. Eleven of 49 C-fibers showed intermittent conduction block before the occurrence of the complete block, whereas 38 of 49 fibers exhibited direct conduction block without showing intermittent firing activities. These results imply a greater variation among C-fibers in the activity-dependent excitability changes, especially in the buildup and recovery of the hyperexcitable phases. This study has been supported by KOSEF grant 891-0700-008-2.
bilateral VB thalamotomy does not reduce pain in the formalin test

SII has the most robust response of the multiple cortical areas activated during painful thermal stimuli in humans, using multiple slice functional MRI. A 1.5 Tesla MRI system with a wide slice pixel size of 1.6 X 1.6 mm, a single surface coil, and an echo planar acquisition sequence were used to image changes in activity in the middle third of the cerebral cortex contralateral to experimentally applied stimuli. In a single experiment, multiple repetitions of noxious heat (45-48°C for 50 s, control = 40°C), motor (sequential application of D-2 to D-4), and mechanical vibratory stimuli (attached to D-1, control = rest) were presented. Eight-10 mm slices were imaged during 6 stimulus-control cycles in each session. Five images/slice were obtained during stimulus or control. Images were superimposed on high resolution MR images.

Noxious heat was sensitive in significant (F2,12 = 6.02, p < 0.05) activation in the primary (SI) and secondary (SII) somatosensory cortices, primary motor (MI) cortex, and posterior area 24. The largest increase in activity occurred in SII where 3.5% increases in intensity over baseline were noted. The activity in MI was larger than that in SI. For the motor task, significant activation was noted in M3 (6% increases), premotor cortex, posterior area 24, SII, and SII. Mechanical vibratory stimulation resulted in increases in activity in SI and SII (2.5% increase).

These results show that multiple cortical areas are activated in response to thermal painful stimulation, including the motor cortex. In addition, the somatotopy of pain perception corresponds with those observed for mecanoeception and motor tasks.


Functional imaging studies in man show that phasic noxious stimuli reliably evoke increased CBF in several brain regions. However, studies using tonic noxious stimuli show consistent results for tonic pain perception. The techniques are not clear. In the present study we tried to maximize detection of tonic pain-related CBF changes by using a regression analysis which weighs experimental conditions according to subjective perception rather than to physical characteristics of the stimuli.

Hypoxia emission tomography (HET) was used to measure CBF following bolus injections of H2O in 11 normal males during 6 conditions (70s immersion of the index finger in 35°-39.5°, 45°, 46°, 49° or 49°C circulating water). A regression analysis was performed across conditions using subjects' psychophysical ratings to model a monotonotic function between perceived pain and CBF. A directed search of cerebral regions of interest, predicted from studies of phasic pain, revealed significant correlation between pain perception in the contralateral sensorimotor cortex (SII), secondary somatosensory cortex (SII), rostral insula (RI), anterior cingulate (AC) and thalamus (3-40). Using SII and RI showed no trend towards activation. A global search of the entire intracerebral volume revealed additional regions of high correlation in areas involved in motor processing such as red nucleus, basis ganglia and cerebellum (3-40), significance threshold adjusted for the larger search area). These data suggest that although tonic pain may be less robust in evoking CBF changes, more sensitive analysis techniques can reveal effects of such stimuli in regions activated by phasic pain stimuli. Supported by Canadian MRC and FRSC.
SPATIAL DISTRIBUTION OF CHRONIC PAIN-INDUCED BLOOD FLOW CHANGES IN THE HUMAN BRAIN. C.R. Coghill, K.F. Bertram, R.H. Gracely, M. Max, M. Byas-Smith, G.J. Bennett, and M.J. Jancan. Neurobiology A. Anesthesiology Branch, NINDS, and Clinical Brain Disorders Branch, NIMH, NIH, Bethesda, MD 20892

Functional brain imaging may provide one tool by which chronic pain syndromes may be evaluated accurately, yet little data exist on the spatial distribution of brain activity during chronic pain. In order to better characterize pain-related changes in the human brain, pain, five patients who underwent functional brain imaging studies. Four patients suffered from post-traumatic chronic pain and secondary hyperalgesia affecting one of their limbs and the painful area was identified with positron-emitting radioisotopes in the trigeminal distribution. All subjects exhibited ongoing pain in the absence of overt somatosensory input. A bolus of 15O H2O was used as an index of functional activity and was measured by 60 s positron emission tomography (PET). Scans following bolus intravenous injection of 15O H2O Subjects underwent multiple PET scans during rest and during alodinia elicited by low intensity mechanical stimulation. Preliminary analyses reveal that these patients demonstrate statistically reliable and robust asymmetry that reflects blood flow distribution during both rest and alodinia. However, these findings, however, are consistent with similar results obtained from cancer patients with unilateral pain (Di Piero, et al., Pain, 46, 9, 1991) and indicate that asymmetry in thalamic blood flow is a salient feature of different types of chronic pain.


PET studies of phasic and tonic experimental pain using regional cerebral blood flow (rCBF) as the index of activity have demonstrated activation of primary and secondary somatosensory areas (SI, SII), anterior cingulate cortex (ACC) and thalamus. We investigated the cerebral representation of chronic ongoing pain in patients with mononeuropathy (MNP). Seven patients (42±3 yrs) with MNP in the lower extremitie (4 in the right, 3 in the left) were recruited. Three PET image sets were obtained with intravenous injection of 15O H2O (30.0 mCi) in the patient's habitual painful state and following a successful regional lidocaine blockade (3 mg/ml) in the painfree state, respectively. Pain intensity was assessed and the actual measurement of rCBF was performed using a common anatomical representation and subsequently normalized to the global mean. The images of the painful and painfree states were subtracted pairwise within the subject and averaged according to the side of MNP. The determination of significant change was based on the local maximum. Significant increase in rCBF was observed, in the painful state, in the right ACA (Brodmann 24) regardless of the side of MNP. Other activated regions included insula, association cortex and cerebellum. Reduced rCBF was recorded in the thalamicus. Indeed rCBF was observed in the SII and SIII. The present results suggest a right hemispheric lateralization of CAC (Brodmann 24) as well as a reduced thalamic activity in chronic pain perception. Experimentally induced pain may contain a more expressed component in the cerebral response (S II) pertaining to the localization of the painful event as well as intensity coding. [Supported by Swedish IRC (R679467)]

464.10 REGIONAL CEREBRAL BLOOD FLOW (rCBF) CHANGES DURING PAIN: AN ANIMAL MODEL T.J. Morrow*, P.P. Daneman, K.A. Frey, and K.L. Casey. Dept. of Neurology, Physiology, Lab. Animal Medicine, Nuclear Medicine, University of Michigan and VA Medical Center, Ann Arbor, MI 48105

BACKGROUND Recent studies of regional cerebral blood flow (rCBF) in humans using positron emission tomography (PET) have suggested new ideas regarding which CNS structures participate in the perception of pain in man. To examine the effects of CNS lesions and pharmacological manipulations on rCBF in pain, an animal model analogous to PET must be developed. The goal of this study was to evaluate the use of a positron emitting radiopharmaceutical, 15O-H2O-PET into and out of the hindlimb served as the nociceptive input. After 5 minutes stimulation, 10 mCi of 99mTc-HMPAO was injected into the catheter. Five minutes later the rats were sacrificed and the brain removed. Twenty microinjection sites were cut and mounted. Standard autoradiographic images of the brain sections were then analyzed for relative optical density. Ipsilateral and contralateral ROI's (regions of interest) were sampled separately from several brain regions, and statistically analyzed (t-test) for differences. RESULTS. This technique showed similar rCBF changes as seen in human PET studies, e.g. side to side differences in sensorimotor cortex (1.84% diff, p<.001) and cingulate cortex (7.58% diff, p<.05). CONCLUSION. Measurement of rCBF promises to be an exciting new tool for examining changes in CNS activation patterns during pain perception in the awake animal brain.


Recent studies have shown that the basal ganglia receive a nociceptive input (J. Neurophysiol., 1993, 69:1890) and play a role in nociceptive input to the ventral lateral nucleus, (Pain, 1985, 23:83). The aim of this work is to investigate the possible role of the neostriatum in the modulation of nociceptive input in rats. The latency and frequency of autotomy (AT) following leg degeneration were compared between a control group (n=6), a basal ganglia lesioned group, in which kainic acid (0.5 µl, 0.1%w) was stereotaxically injected in the neostriatum one week prior to the leg degeneration (n=14), and sham group in which scalp overlying the skull was sectioned and sutured one week prior to the leg degeneration (n=6). All rats in the control and sham groups exhibited autotomy (AT) with a mean frequency of less than 10.3 ± 0.8 days and 10.3 ± 0.8 days, respectively. Only 5 out of the 14 (35.7%) neostriatum-lesioned rats exhibited AT which was also significantly decreased in onset to 24.6 ± 0.7 days. These results suggest that the neostriatum may play a role in either in processing sensory input or in the ability to locate and orient the head away part of the body originating the pain sensation. (Supported by grants from the Diana Tamari Sabeag Fund and the University Research Board).

464.12 POSTURAL TUNING OF CUTANEOUS NOCICEPTIVE INPUT TO A SPINAL MOTOR SYSTEM M. MILLER*, H. AND SCHUINDLICH, J. Department of Physiology and Biophysics, Lund University, Sölvegatan 19, S-223 62 Lund, Sweden

Detailed information about the development and function of nociceptive systems is necessary for understanding the mechanisms by which the adult organization of these systems is accomplished. Also, such information is needed for adequate interpretation of the responses to potentially noxious stimuli. We have now examined the postural development of the rat withdrawal reflex (WDR) evoked by noxious thermal stimulation (CO2 laser-pulse) in decerebrated, spinalized rats. Behavioral studies in intact rats showed stereotyped, often misdirected reflex withdrawals, indicating immature properties of the HWR system at this age. WRR-recordings in rats examined at postnatal day (PND) 10-21 revealed characteristic withdrawal reflexes (WRFs), typically covering the whole plantar surface, small response amplitudes and great response variability. With increasing age a decrease in RF size and an increase of response amplitudes were observed in the extension digitus longus, peroneus tertius posterior, whereas responses evoked in gastrocnemius-soleus decreased with age and were absent after PND 21. At PND 21-25 the WRFs of the muscles studied were similar to their counterparts in rats, although detailed distribution of sensitivity still differed somewhat from the adult. According to our data the final tuning of cutaneous WRF of the nociceptive withdrawal reflexes occurs after the third week of life. We suggest that an experience-dependent process is involved in this tuning.

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645.1
CHOLINERGIC INPUTS ONTO DIRECTIONAL GANGLION CELLS. C. Brandon, Dept. Cell Biology and Anatomy, Chicago Medical School, North Chicago, IL 60064.

In the retina retin, ON-Center, Directionally-Selective neurons (ON-DSCGs) project to the accessory optic system of the midbrain. The directional component of their receptive fields appears to be generated via GABAergic input and modulated by cholinergic input. The dendrites of ON-DSCGs intermingling with the processes of starburst amacrine neurons (SBANs; 90), current work examines the synaptic connections between these two cell types.

ON-DSCGs were labeled by retrograde transport from the medial terminal nucleus, and individual labeled cells were injected with Lucifer Yellow in vitro. The tissue was stained immunocytochemically for both Lucifer and choline acetyltransferase (ChAT), and tangential sections through the inner plexiform layer were examined by EM. By light microscopy, ON-DSCG dendrites were completely embedded within the plexus of starburst amacrine dendrites. By EM, ON-DSCG dendrites were surrounded by an extremely dense plexus of ChAT-IR processes; they received many direct synaptic inputs from these processes, which covered virtually their entire surface area. The bulk of the remaining input was from very long, en passant synapses from non-ChAT-IR amacrine processes; these had a very low electron density.

Identified ON-DSCG therefore receive dense, monosynaptic cholinergic input. The large, non-cholinergic synapses, because of their size and placement, seem ideally placed to veto the massive, excitatory, cholinergic input that impinges on large dendritic branches of the ON-DSCG.

Supported by NIH/BRSG Grant 507 RR0366-28.

645.3
REGULATION OF GLUTAMATERGIC TERMINALS IN THE INNER PLEXIFORM LAYER OF THE TIGER SALAMANDER RETINA. D. Hedener, W. Yu, and R. F. Miller, Department of Physiology, Graduate Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455.

Using a retina slicing preparation, we examined the effects of different neurotransmitter agonists/antagonists on evoked synaptic currents in inner retinal neurons of the tiger salamander. Synaptic currents were evoked by light or by a pulse of hyperosmotic sucrose applied to the IPL. Synaptic currents were monitored through whole cell voltage clamp recordings from neurons in the ganglion cell layer. In the presence of picotoxin and strychnine, GABA_A agonists reduced sucrose evoked glutamatergic synaptic transmission in approximately half of the cells studied, and in some cases reduced the synaptic currents more transiently. In almost all cells studied, GABA_A agonists inhibited light evoked synaptic transmission. The results of this study are consistent with the idea that GABA_A receptors inhibit transmitter release from glutamatergic terminals in the IPL. The results of this study also suggest that local hyperosmotic stimulation of transmitter release from terminals in the IPL can be used to study presynaptic regulation of transmitter release.

(Work supported by NIH Grant: EY-00844 to RFM).

645.4
VOLTAGE-CLAMP AND CURRENT-CLAMP RECORDING FROM RETINAL GANGLION CELLS. G.M. Ratto, L. Lambardi, S. Bizzi, L. Crepato and L.M. Chalupa. Ist. di Neurobiologia CNR Fns, Italy and Center for Neuroscience, University of California, Davis CA.

The electrical properties of retinal ganglion cells (GCs) were analyzed in rat retina slices using the patch clamp technique in the whole cell configuration. The cells were identified morphologically by filling them with diffusible dyes.

Two classes of GCs were identified on the basis of the characteristics of their potassium current. In one class, the K current had two components: a transient rapidly activating current and a slowly inactivating current. In some cells the slow component lasted for several hundred ms without appreciable inactivation. In the second class of cells the transient current was absent.

Current clamp recordings also provided evidence for two distinct classes of cells. In one group of GCs the onset of a steady depolarization caused by outwardly injected current evoked a single large spike, occasionally followed by a few spikes of decreasing amplitude. In these cells no sustained discharge was observed upon injection of constant current. In the second group of GCs the current injection evoked a sustained discharge whose frequency and amplitude increased with the extend of depolarisation. At the largest levels of depolarisation these cells produced transient discharges superimposed on a gradually increasing depolarisation which followed a time course similar to the closure of the slowly inactivating potassium current. The sustained cells displayed spontaneous activity, with spike frequency varying with relation to the baseline potential.

Supported by grants from NATO and CNR (bilateral project Italy-USA) and a NIH Fogarty International Fellowship (L.M.C.).

645.5

Presumed ganglion cells of the Xenopus retina were studied in short term culture by the whole-cell version of the patch clamp technique. The studied neurons were identified by their characteristic current-voltage relation. In a cocktail of TEA (20 mM), 4AP (5 mM), TTX (1mM) and Ba (20 mM) as a charge carrier, we observed both voltage-activated calcium (and high voltage-activated (HVA) Ca currents. The HVA current was blocked by nifedipine (0.1uM) and enhanced by dopamine (50uM). LVA current was blocked by Ni (20 uM) but was not affected by nifedipine. The metabotropic glutamate receptor antagonist (ACG) reduced the amplitude of the HVA Ca current by 46.62% (n=7). The inhibition by ACP increased to peak within 20s and was completely reversed within 15s by a Ringer wash.

The involvement of G-protein in the inhibitory effect of ACP was studied by including GDP[7S] (300uM) in the patch pipette, resulting in a significant reduction in ACP-induced inhibition. In contrast, the inhibitory effect of ACP on Ba current was irreversible when GTP[yS] (200uM) was added to the patch pipette. In addition, internal application of GTP[yS] by itself reduced only the HVA component of the Ba current. These results indicate that activation of G-protein-coupled metabotropic receptors on ganglion cells by glutamate can lead to the modulation of voltage-gated Ca current. Supported by NIH grant EY03570 to P.W.

645.8
MICROGLIAL AND MACROPHAGIC RESPONSE IN RAT RETINA FOLLOWING OPTIC NERVE SECTION. S.C. Sharma, R. Garcia-Valenzuela, Dept. of Cell Biology, New York Medical College, Valhalla, NY 10595.

Cellular debris, following retrograde degeneration of retinal ganglion cells (RGC), has been shown to be cleared by resident microglial cells in the retina (Thanos, 1992). Here we show that blood derived macrophages (MΦ) have a distinct role in the removal of RGCs' debris. The phagocytic process was studied using double labelling with antibodies against macrophages and phagocytic cells (ED-1 and OX-42) together with retrograde tracing of RGCs after application of either a hydrophilic dye (Fast blue) or a membrane bound dye (Di I). It was found that while resident microglia from different retinal layers take care of most of the debris, macrophages invade the retina primarily in the nerve fiber layer and are probably dedicated to engulf axonal debris since unlike microglia, they stain only with Di I and not with the membranous and topographic aspects of these phenomena were also studied.
645.7


A small lesion at the vitreal surface of the retina produces degeneration of retinal ganglion cells whose axons are severed and leaves a small region in the retina depleted of ganglion cells. When the lesion is performed at an early stage during postnatal development the regeneration of the ganglion cells in the boundary of the depleted area show an abnormal elongation of the dendritic trees toward the bare area. A possible explanation for this is that dendrites compete either for synaptic space or chemical factors. Alternatively one may suppose that the electrical activity plays a role in the regulation of dendritic arborization as it happens in the segregation of retinal afferents at the LGN level. In the present study we combined a small retinal lesion with the block of the regenerative electrical activity by intracocular administration of tetrodotoxin. This treatment was continued for the whole critical period. The LGN were then injected with HRP and the retinae were wholemounted and reacted. The ganglion cell population at the border of the depleted zone was then analyzed. It is seen that neurons near the border present a symmetrical dendritic arborization with few exceptions located in correspondence with the lesion. These results suggest that the electrical activity plays a role in shaping the dendritic arborization during the development of ganglion cells.

645.9


Following injury, most central neurons in mammals are unable to regenerate, even after the manipulations of their cellular and molecular environment. Modifying their intrinsic functional properties though gene transfer might be necessary for complete restoration of their anatomy. Here we describe a functional transsection of axotomized retinal ganglion cells by immediate administration of plasmid at their cut ends. Two different plasmids were used, containing either the SV40 promoter linked to the luciferase gene, or the CMV promoter linked to the LacZ gene. Assays for the expression of both reporter genes demonstrated that a significant proportion of ganglion cells express them successfully 3 and 6 days after retrograde transport. Such an approach might be useful in studies of neuronal molecular functions in vivo, and as an experimental therapeutic strategy.

645.11


Soma and dendritic field size of retinal ganglion cells (RGC) are not sufficient criteria to separate clearly the cell types described by Perry (1979) and Dreher et al. (1983). We therefore quantified dendritic morphology of RGC to allow unequivocal classification of different RGC types. 50 RGC, identified by retrograde labeling, were injected intracellularly with lucifer yellow and morphometrically analyzed. As previously described, we were able to classify three cell types by soma and dendritic field size. This classification is not sufficient because we could not identify these cells as a particular cell type. For example, type I neurons, with soma area ranging from 200-500 μm², can be distinguished from type II neurons (50 and 150 μm²). Type III neurons however are as a similar soma size than the type I. We therefore tried to find additional criteria for RGC classification using quantitative analysis of dendritic morphology. These include: dendritic spread, dendritic complexity at growing point, and soma, or segment length in concentric ordering. Three cell types can be clearly characterized by average segment length (ASL), which is shorter in type II cells, intermediate in type I cells, and longest in type III cells. In type II dendrites the ASL is 12 μm in all branching orders up to 13th order dendrite, thereafter shorter. Especially in type III dendrites the 1 and 2 order segments of the centripetal order (end segments) are very long (40-50 μm) in type I dendrites the segments of the 1. order dendrite are relatively short (25 μm), the segments of the 2 to the 7 order very long (40-50 μm) and thereafter extremely short (10-20 μm). By contrast the ASL segment order, dendritic field size and soma size all three cell types can be classified unequivocally. There is indication of further subgroups of cells types in all three cell groups.

645.8

SELECTIVE BLOCKADE OF MULLER CELLS GLUTAMINE IN CAT RETINA CAUSES OCULAR DOMINANCE SHIFTS IN VISUAL CORTEX. B.R. Robinson, K.M. Lee, M.G.P. Rosa, L.M. Schwindt, and D.I. Vaney, Vision, Touch, and Hearing Research Centre, Department of Physiology and Pharmacology, University of Queensland, St. Lucia, Australia, 4072.

Inhibition of the enzyme glutamine synthetase in Muller cells by methionine sulfoximine (MSO), causes a complete loss of glutamate immunoreactivity in retina and visual cortex. This study investigates changes in response properties of neurons in cat visual cortex following intracocular MSO injection. Adult cats were anesthetised with thiopentone sodium (40mg/kg IV) and N20/02 (70:30), while pancuronium bromide (Pavulon 0.15mg/kg hr IV) was administered to minimize eye movement. The eyes were injected with 1.0 ml of MSO at a dosage equivalent to 2ml. Multiple electrophysiological responses were recorded from visual cortical areas V1 and V2 over a period of 24 hours. With both eyes open, the histograms for ocular dominance exhibited a slight shift towards the un injected eye. This shift was statistically significant (p<0.05) for the V1 area. The changes were seen in both hemispheres, and in both V1 and V2; the shift being greater in V2. The cats were subsequently euthanised with sodium pentobarbital (60mg/kg IV) and their retinas processed for cytochrome oxidase. The results showed that loss of glutamate, while the ganglion cells and bipolar cells were depleted of glutamate. These data lead us to conclude that Muller glial cells are an essential component of gluta..matergic neurotransmission in the primary visual pathway.

645.10


We investigated the localization of the mRNAs of γ-Aminobutyric Acid A receptor α1 subunit (GABA A α1) and L-Glutamate Decarboxylase mRNA (GAD) among subclasses of RGCs by non-radioactive in situ hybridization. Adult male Wistar rats were used after overdose injection of pentobarbital. cRNA probes of GABA A α1 and GAD were labeled with Digoxigenin-11-UDP (Boehringer) and hybridized with vertical sections of retina. Soma diameter measurement was performed for all cells with positive signals in the ganglion cell layer (GCL). GABA A α1 and GAD mRNAs were detected in the inner nuclear layer and the GCL. In this work, we classified the cells with soma diameter larger than 13μm as RGCs (Perry, 1981, Beale and Osborne, 1982). Since signal positive cells for GABA A α1 mRNA distributed within 5.5-25μm in diameter, RGCs including α-cells (217μm) expressed GABA A α1 mRNA. Soma diameter of cells expressing GAD mRNA distributed within 6-16μm in diameter. We interpreted to indicate that small (S) and medium-size (M) RGCs but no α-cells expressed GAD mRNA. 

645.12


The retina has previously been shown to have a projection to the suprachiasmatic nuclei (SCN). SCN neurons have been shown to have a maintained firing rate that either increases or decreases in the firing rate in response to increased illumination (Gros and Mason, J.Comp.Physiol., 1980, Meijer et al., Brain Res., 1986). The goal of this research is to characterize the retinal ganglion cells to determine which neurons are most likely to play a role in the photic entrainment of circadian rhythms. Fluorescently labeled latex beads were stereotactically injected into the SCN of rabbits. After a recovery period of one week, we were able to label ganglion cells were isolated and mounted in a fluorescence microscope and superfused with oxygenated Ames medium. The activity of the labelled cells was examined with extracellular recording while visual stimuli were projected through the microscope condenser. The cells were then intracellularly impaled with a pipette electrode and injected with HRP and processed to allow morphological examination. The first cells labelled exhibit a sustained rate of firing that tonically increases with an increase in general illumination, with no sign of an antagonistic surround. These cells respond to small patches of MSO agarose, and their spontaneous activity is consistent with a role of luminance coding. The cell's morphology is characterized by small somata and very fine dendrites that are bifurcated. Labelled cells were located in the inner plexiform and outer nuclear layers, and are consistent with axonal arbors extending to the inner nuclear layers, and are consistent with a role in the photic entrainment of circadian rhythms.

Supported by RMPT: Project 07NBL04
645.13

DISTRIBUTION AND COVERAGE OF BETA CELLS IN THE CAT RETINA

We have estimated the distribution of beta cells in the cat retina by
mapping the densities of medium sized (non-alpha) cells labeled by
retrograde tracer deposits largely or completely confined to the geniculato-
A-layers. We visualized the dendritic fields of beta cells by intracellular
staining in vitro. In the nasal retina, beta cells exhibited a well-developed
visual streak and, except centrally, accounted for about 40-50% of ganglion
cells. However, the incidence of beta cells increased substantially in the
central retina (to about 60-70% of all ganglion cells; see Neuron. Abstr.
15:292, '80) and throughout much of the temporal retina (to about 70%).

Though beta-cell densities were lower temporally than nasally, the distribution
was far more symmetrical about the line of nasotemporal division than the
distribution of all ganglion cells. These results are consistent with our
observation that colliculopetal W-cells (which account for the vast majority of
non-beta cells) exhibit a complementary distribution: they make up about half
of the ganglion cells in the mid- and far-peripheral nasal retina, including the
visual streak, but are underrepresented in the area centralis and temporal
retina (where they make up, respectively, about 33% and 25% of all ganglion
cells). Dendritic-field areas of beta cells systematically reflected local beta-
cell density. For example, in the nasal retina (eccentricity: 30°), areas were 4-
fold smaller within than outside the visual streak. Coverage factor (beta-cell
density X dendritic-field area) remained constant everywhere, whether central
or peripheral, nasal or temporal, or on or off the visual streak (mean = 3.0; SD
= 0.76; N=200). We suggest that the beta-cell system shows greater binocular
balance and is more specialized for fine sampling of central visual fields than
other cell classes, and has enhanced functional capacity along the horizon.

Supported by NIH EY08108.

645.14

THE SPECTRAL SENSITIVITY OF GANGLION CELLS IN THE
MOUSE RETINA VARIES ALONG THE INFERIOR-SUPERIOR
AXIS. E.B. Soucy, E. Wu and M. Meister* Dept. of Cellular and
Molecular Biology, Harvard University, Cambridge, MA 02138.

We are interested in the nature of the information encoded in the
spike trains of ganglion cells. Using a multi-electrode array, we recorded visual responses from the population of mouse retinal
ganglion cells. The response properties of each cell were determined by computing the reverse-correlation function of its spike train to a
pseudo-random stimulus. This allowed us to measure the spatial,
temporal, and spectral components of an individual ganglion cell's receptive
field. Initial characterization of mouse retinal ganglion cells revealed a relationship between spectral sensitivity and retinal
location, with cells in the inferior region displaying greater
sensitivity to short wavelengths relative to cells in the superior
region of the retina. This was consistent with the cone ratio of mouse retinal
ganglion cells.

Supported by Lucille P. Markey Scholarship to M.M., and a NSF
Pre-Doctoral Fellowship to E.W.

645.15

TWO REMARKS ON CODING IN THE OPTIC NERVE OF
Of Biomedical Engineering, Rutgers Univ., Piscataway, NJ 08855,
and Lab for Computer Science, MIT, Cambridge, MA 02139.

We studied pulse interval coding on two types of retinal
ganglion cell (RGC) in the frog, Rana pipiens, by recording extracellularly from single optic nerve fibers with metal-filled
microelectrodes. Records were taken from color sensitive fibers
(RGC Class V) and "dimming detectors" (RGC Class IV).

With regard to the color fibers, we noted strikingly different
pulse interval patterns on a single fiber in response to light stimuli of
two different wavelengths, despite approximately equal average
firing frequencies in each response. These data are consistent
with a pulse pattern rather than pulse frequency coding for color.

The dimming detectors had a sensitivity to step changes in
light intensity of less than 0.01 decade, and a sensitivity to
continuous changes in intensity of less than 6 decades per hour.
The evoked response to step decrements in light intensity was
logarithmically related to the magnitude of the step, and was
independent of background illumination over a range of at least
two decades.

645.16

INITIAL PHASE OF ROD RESPONSES, GANGLION CELL
FIRING AND BRIGHTNESS SENSATIONS CORRESPOND
UNDER DIFFERENT BACKGROUND CONDITIONS.
K. Djupsund1, K. Donner1, N. Pyrhönen1 and T. Hariyama1,2,1Dept.
Zoology, FIN-00014 Univ Helsinki, Finland and 2GISIS, Tohoku
Univ, Sendai 980, Japan.

The rods are the first filter in the visual sensory chain and ganglion
cells (GC) the last retinal processing step before visual information enters the optic nerve and higher processing. Therefore one might in
these nerve cells find common constraints and guidelines for processes mainly studied in psychophysics.

We have compared on one hand brightness sensation and adaptation
data and on the other hand the timescale of the rise of intracellularly recorded rod responses and extracellularly recorded GC response frequencies in the frog Rana temporaria to flash/step stimuli.

We show that if brightness sensations are determined by the initial
rise of the photoreceptor signal, this can account for the intensity-
brightness sensation relationship under different backgrounds.

The rise of rod responses accelerated with a power of ~0.14-0.2 over sub-
saturating background and stimulus ranges, which corresponded to the rate of GC responses.

646.1

CHOLINERGIC DEAFFERENTATION OF THE VISUAL CORTEX
BY INTRACORTICAL INFUSIONS OF 192 IGG-SAPORIN IN RATS:
EFFECTS ON VISUAL DISCRIMINATION AND VISUAL
ATTENTION. M. Sarter*, L.A. Holley and M. Matej. Dept. Psychology,
Ohio State Univ., Columbus, OH 43210.

The visual cortex, as all cortex, is innervated by the cholinergic neurons
situated in the basal forebrain. The functions of the cholinergic afferents of
this cortex have remained unclear. Furthermore, the role of the cholinergic cell
loss in the visual dysfunctions of patients with senile dementia is
unknown. Rats were trained to discriminate between simultaneously
presented pairs of visual stimuli flashing at 5 Hz versus 4.17, 3.75, 2.5,
1.67, or 1.25 Hz. Cholinergic inputs to the visual cortex were selectively
lesioned by infusions of the immunotoxin 192 IgG-saporin into this area
(0.01 µg/0.8 µl per injection). Control animals received either infusions of
the immunotoxin into the frontoparietal cortex or infusions of vehicle into
the visual cortex. Lesions performed after the animals learned the task did
not robustly affect performance. Lesioned placed before the acquisition of
the task resulted in a transient impairment in the rate of acquisition. The
role of cholinergic afferents of the visual cortex in the animals' ability to
detect visual signals presented for 25, 50, or 500 msec and to discriminate
these signals from non-signal events was also examined. These experiments represent a first step toward the determination of the functions
of cholinergic inputs to the visual cortex.
NEUROCHEMICAL EFFECTS OF MONOCULAR APHASIA IN SUBPRAGNULAR LAYERS OF V1 OF THE ADULT MACAQUE. S.S. LeDoux and R.K. Carder. Zanvyl Krieger Mind/Brain Institute, Johns Hopkins University, Baltimore, MD 21218.

Unlike monocular enucleation or intravital TTX injections, monocular aphasia results in a chronic defocusing without eliminating retinal output. Loss of neural activity in one retina, as with the other forms of monocular deprivation, leads to chemical changes in V1. In the present study, cytochrome oxidase (CO) histochemistry and calbindin immunocytochemistry were used to determine if chronic defocusing without elimination of retinal input can lead to similar neurochemical changes in the subpragnular layers of V1. CO and calbindin staining are normally complementary, with CO staining occupying puffs along the centers of both sets of ocular dominance columns, and calbindin staining occupying the surrounding CO-poor interpuffs. After 3-12 weeks of monocular aphasia, changes in CO histochemistry and calbindin immunocytochemistry are apparent. CO-rich puffs at the centers of normal-eye puffs fuse to form stripes while CO-rich puffs at the centers of deprived-eye columns become pale and shrunken. Calbindin immunostaining, normally intense in the CO-poor interpuffs and weak in the CO-rich puffs, is similarly affected. Alternating and lightly stained stripes are centered respectively on the stripes of CO-rich puffs of the normal-eye columns. These results are similar to those found in TTX-injected monkeys indicating that aphasic amblyopia also produces neurochemical changes across compartments and ocular dominance columns. Supported by ET/06344 and FR8Q.

ONCOGENE EXPRESSION REVEALS OCULAR DOMINANCE COLUMN IN ADULT CAT VISUAL CORTEX. L. Aronson, M. P. Watanabe, W. Vanduffel, F. Vandenberghe, and D. G. Harts. Brain Research, 544, 113-120.

We used immunohistochemistry to determine whether manipulation of visual input can induce changes at the protein level of two oncoproteins, c-fos and zif-268, in the primary visual cortex of the adult cat. Under normal visual conditions, c-fos and zif-268 were expressed at low basal levels in layer II/III, 1/8 and VI of areas 17 and 18, while c-fos was hardly detectable. Monocular deprivation drastically influenced the expression of both oncoproteins. Recently after one hour of monocular visual stimulation, Fos and zif-268 positive nuclei were distributed in a columnar fashion. Using longer monocular deprived cats and cytochrome oxidase histochemistry, we demonstrated that the immunocytochemically detected columns are ocular dominance columns corresponding to the stimulated eye. From immunocytochemical double stainings we know that both oncoproteins are expressed in the nuclei of non-GABAergic neurons. Supported by IUPAP Vision & Memory.


We previously constructed a model visual cortical circuit with short-range (<300μm) intracortical excitatory and inhibitory connections which yielded emergently sharp orientation selection (Sur et al., 93). The ability of this circuit to orient tuning from direct inhibitory inputs to single cells, but a substantial contribution from the distributed inhibitory inputs to a cortical column. Intracortical excitatory connections are anisotropic, conveying responses to preferred stimuli, and inhibit primarily the gain of excitatory feedback. Here we demonstrate that cortical gain also depends on stimulus contrast: the gain rises to boost mild suprathreshold stimuli and drops off at high stimulus contrast. This mechanism yields cortical contrast response functions that rise more rapidly and saturate more quickly than their monocular inputs.

We have added to our model two forms of longer range cortical connections: very sparse inhibitory input from oblique or cross orientations (300 - 600μm) and more extensive excitatory input from longer ranges (2-10mm). Long-range inhibitory connections comprise less than 20% of inhibitory and less than 5% of all model synapses. Although long-range inhibitory connections are not required to achieve sharp orientation tuning, these connections permit the circuit to maintain sharp orientation selectivity and contrast gain control across the full range of suprathreshold stimulus contrasts (Skottun et al., 87). As in the short-range model, inhibition is strongest at the preferred orientation and has progressively distributed rather than directed effects on tuning. Adding long-range excitatory connections produces biphasic effects that depend on stimulus contrast. Strong iso-orientation surround stimuli can up- or down-modulate the responses to stimuli within the classical receptive field: responses to subthreshold or weak center stimuli are amplified, while those to strong center stimuli are reduced. Supported by McDonnell Fund, MH10671, EY05635, and EY07023.


Attempts at understanding the functional relationships between cortical micromicroscopy and receptive field generation have been hampered by the technical difficulty of analyzing the biophysics and synaptic physiology of cortical neurons in vivo. Preparations have circumvented this difficulty by using an in vitro preparation of the eyes and brain of freshwater turtles, allowing us to record intracellular from cortical neurons and to present natural visual stimuli to the eye cup. Results from intracellular recordings have been combined with anatomical and with compartmental models of cortical neurons and of neural circuits implemented with the Nodus software package. We investigated the responses of pyramidal cells in the visual cortex to 1 sec, 660 nm light flashes with intensities varying from 0 to 1500 photons/cm2/sec, finding that threshold light intensities produced small EPSPs. Shape parameter analysis indicated that EPSPs recorded at the soma are usually composite EPSPs. Simulations showed these composite EPSPs could result from asynchronous activation of geniculate boutons on several dendrites of the cell, consistent with earlier results from our lab using electrical stimulation of geniculate afferents. Supported by PHSG Grant 88-3852.


Residual visual capacity in humans with damage to striate cortex has been reported to include sensitivity to presence and direction of motion (e.g., Pererin, 1991, Neuronuvs, 2:391). We investigated the ability of three monkeys with large unilateral striate lesions to process motion in the affected portion of the contralateral visual field. Lesions were made at 5 weeks of age in two animals and in adulthood in the third. Stimuli were small (5 deg. diameter) gaussian-filtered fields of dynamic random dots (dot size 0.1-0.5 deg.) presented on a video monitor. Moving stimuli contained 98% correlated motion. A go/no-go paradigm employing a saccadic eye movement response was used to test the ability of each monkey to discriminate 1) upward from downward motion, 2) speeds of 4 and 0 deg. /sec., and 3) upward motion from motion noise (0% correlated motion) and 3) upward motion from a static field of random dots. All animals quickly learned all discriminations in the intact hemifield. In the impaired hemifield, however, they failed to perform above chance on all tasks except the discrimination of moving vs. static dots. The results indicate that preserved visual capacity after striate cortex damage in monkeys does not include at least some types of motion sensitivity, i.e., the integration of local motion signals present in dynamic random dot displays. Supported by NSF BNS-9109743 and NIH MH-19420.

STUDIES OF HUMAN VISION COMBINING MEG AND ANATOMIC AND FUNCTIONAL MRI. J.S. George, C.J. Ains, J.C. Mosher, J.A. Schlotz, C.C. Wood, J.D. Levine and J.A. Saunders. 1 Los Alamos National Laboratory, 2 Albuquerque Federal Regional Medical Center. Integrated analysis of human anatomic and functional images offers a powerful tool for human brain mapping. MEG and EEG provide excellent temporal resolution of neural population dynamics. MRI provides excellent spatial resolution of hemodynamic changes, and their combination allows for an alternative measure of functional activation. These methodologies constrain and complement each other and can thereby improve our interpretation of functional neural interactions. In these experiments, MEG data (317 channels) and MRI data (6 slices, 8 or 10mm thick, with 2 mm in-plane resolution) were collected using a patterned stimulus in the lower right visual field. T1 weighted 3-D images of the blood flow were acquired during the exposure. The geometry of cortex and of major conductivity interfaces within the head was defined by segmentation of MRI data using semiautomatic image analysis procedures in MIRview, a software package developed in our laboratory. Cortical edge voxels were identified and cortical normal vectors were calculated for edge voxels using a 3-D convolution over the 26 nearest neighbors. 35-50,000 voxels were required to define the cortical surface at 2 mm isotropic resolution. Conductivity boundaries were defined by stain-wraping an isosurface-based mesh over the surfaces. Electric potential was evaluated at nodal points using a boundary integral calculation. Magnetic fields at sensors were calculated by evaluating an integral over all node potential values. As a first estimate of MEG sources, the MUSIC metric was evaluated for voxels under the sensor array, assuming locations and orientations derived from anatomical MRI. In agreement with previous dipole-based studies of normal MEG data, at least three sources are evident in these images, a primary response (Y1/12) and bilateral sources near the parietal/occipital border. As a second step, dipole locations were identified based on the observed field, dipole orientations were determined using a simple least-squares fit. Component dipole sources were assigned locations and strength based on MEG and orientations based on anatomical MRI. Such geometrically complex composite sources can be treated as single sources for temporal analysis based on MEG.
DISTRIBUTIONAL DIFFERENTIAL OF GLUTAMATERIC SYNAPSES BETWEEN CYTOCHROME OXIDASE (CO)-RICHPUFFS AND CO-POOR INTERPUFFS IN PRIMARY VISUAL CORTEX OF MONKEYS. E. Nie, R. Curtis and M. Wong-Riley, Dept of Cellular Biology & Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226

Glutamate is a major excitatory neurotransmitter in many regions of the brain. This study aimed to characterize the distribution of glutamate-positive asymmetric synapses (presumed excitatory) than interpuffs. In the present study, a double labeling technique combining cytochrome oxidase with glutamate was used to determine the distribution of glutamate-positive synapses is correlated with a difference of CO activity between puiffs and interpuffs at the EM level. Our results showed that CO activity in glutamate-positive pyramidal axonal terminals. Quantitative analysis demonstrated that there were more glutamate-positive synapses in puffs than in interpuffs. These differences in CO activity between puiffs and interpuffs were detected using 100 μm 3PO (P<0.005). Furthermore, glutamate-positive axonal terminals in puffs contained a higher proportion of darkly stained mitochondria than those in interpuffs, indicating that they might be more active in puffs. In addition, glutamate-positive axosomatic synapses selectively targeted GABAergic neurons, which contained more darkly reactive mitochondria. This study suggests that glutamatergic synapses play an important role in excitatory transmission within puffs and interpuffs. Glutamate may be the neuromodulatory basis for higher metabolic demands related to greater depolarizing activity in puffs than in interpuffs. (Supported by NIH grants EY05439 and NS18122).


The steady-state visual- evoked potential (VEP) of an ordinary pattern-reversal grating contains a robust second harmonic (2H), but little or no fundamental (1H) activity. Cortical 1H processing is of considerable interest, because it is thought that only 1H is clearly linked to neural substrates carrying spatial phase information, which is necessary for the binocular processes of fusion and suppression. Incorporating several simultaneous modulation rates (e.g., F1, F2) enables inferences about cortical processing of 1Hs from nonlinear intermodulation (IM) crosscorrelations of the 1Hs (e.g., F1-F2, F1-F2). Steady-state VEPs were recorded (2z to right ear) from 17 stereorandom (Randot E) adults. Stimuli were 0.86/deg gratings having a total of 60-sec trials. Stimuli contained up to three simultaneous modulation rates (F1=6.1, F2=8.5, F3=12.8 Hz) with monocular, binocular or dichoptic conditions achieved using liquid crystal lenses synchronized at 256/sec monitor frame rate. Fourier analysis extracted amplitudes at frequencies of interest. Monocular IM components, but not monocular 2Hs, were significantly reduced when a different temporal frequency was added to the other eye. Therefore, in these conditions, 1Hs, but not 2Hs, seem to index binocular processing. Nonlinear models of visual processing are discussed in relation to these findings. The Dorothea Haus Roz Foundation.


A well-known feature of the retinocortical projection is that in angular terms the fovea is overrepresented in the striate cortex (retinal ganglion cell size). Recent evidence indicates that the following description of the magnocellular and parvocellular subdivisions of the retinocortical projection. A retrograde, transneuronal tracer (WGA-HRP) from cortex to retina was used to label axons leaving the retina. In this study, we examined the projections of neurons in the magnocellular and parvocellular subdivisions of the retinocortical projection, which is that it could arise simply because the two (parvocellular and magnocellular) retinal maps, represented by populations of ganglion cells with different distributions, are expanded to different extents in order to preserve the topographic co-registration of the inputs to the striate cortex. It suggests that the expansion subserves some visual function.

ANATOMICALLY BASED NEURAL NETWORK MODEL FOR GENERATION OF ORIENTATION SPECIFICITY IN MONKEY PRIMARY VISUAL CORTEX (V1). Q. Wu, J.S. Lund and P.Hadingham, Inst of Ophthalmology, Univ. of London, London EC1V 9EL, and Dept. of Computer Science, Univ. of Western Australia.

To understand the correlation between structure and function in primary visual cortex (V1) of primates, we are attempting to develop a neural network model which is closely based on anatomical observations and aimed at simulating physiological results. Our previous model studied the primary visual evoked response in V1 and V2 in monkeys that spiny stellate cells in V1 accomplish weighted summation of distinct input sets from LGN through dendritic overlap. To simulate physiological results and may also apply to the issue of generation of orientation specificity in V1. Human psychophysical studies demonstrated that the orientation input sets of neurons are produced by a single stimulus oriented stimulus of the orientation input sets of neurons at orientation. Based on this evidence, we propose that orientation specificity in primate V1 is generated in two stages: the first stage is the generation of the two tuning functions centered around horizontal and vertical orientations, and the second stage uses these functions to produce a continuum of all possible orientations. In our model we incorporate the lateral spread of axon collaterals from spiny stellate neurons in V4 as the anatomical substrate for producing the two orthogonal orientations, and the second stage uses these functions to produce a continuum of all possible orientations. Compared to the Hubel and Wiesel model of the generation of orientation specificity, our model is more economical in terms of neuronal wiring and has anatomical, physiological and psychophysical support. Supported by MRC G9206378N and NIH-EY10021.
MONOCLONAL ANTIBODY CAT-305 LABELS GENICULOCORTICAL CONNECTIONS IN PRIMARY VISUAL CORTEX OF CAT, MONKEY, AND FERRET. S. Hockfield* C. Blakemore and J.C. Kind, Section of Neurosurgery, Yale Univ. Sch. of Med., New Haven, CT 06510 and JLin. Lab. of Phys., Oxford, UK. Monoclonal antibody Cat-305 labels cortical layer IV of areas 17 and 18 in the cat (Cerebral Cortex, 1994). This restricted localization suggested that the Cat-305 antigen may be associated with the presynaptic terminals of axons originating in the dorsal lateral geniculate nucleus (dLGN). To address this possibility, we have now examined Cat-305 staining in the cortical areas of macaque monkey and ferret, and in the cortex of cats with lesions in the dLGN.

As in the cat, Cat-305 staining is also restricted to primary visual cortex in the alitferret and monkey. In the ferret, immunoreactivity appears as a dense band of fiber through layer IV in areas 17 and 18. In macaques, Cat-305 labels two bands, both of which are restricted to striate cortex. Laminar analysis demonstrates that the two superficial bands of staining demarcate layers IVa and the deeper band layer IVc, corresponding to the laminar remnant of the geniculocortical afferent.

To determine whether the Cat-305 antigen is associated with or dependent on presynaptic elements in layer IV, unilateral bilateral acid lesions were made in the dLGN of adult cats. Three weeks following the lesion, Cat-305 immunoreactivity was markedly reduced in the cortex ipsilateral to the lesion compared to the contralateral, non-lesioned hemisphere and to normal controls. Staining for monoclonal antibody Cat-301 and GFAP in layer IVa were not affected by the lesion, indicating that there was little or no degeneration of cortical neurons. The decrease in Cat-305 immunoreactivity in cortical layer IV after dLGN lesions suggests that the loss of the Cat-305 antigen is a result of degeneration of the presynaptic terminals of geniculocortical afferents. (Supported by EY0561, MRC, UK, and Oxford McDonnell-Pew Centre for Cognitive Neuroscience)

**VISUAL PSYCHOPHYSICS AND BEHAVIOR II**

**ASSIMULATION OF VISUAL ABILITIES IN ALZHEIMER'S DISEASE AND MENTAL RETARDATION.** S.A. Sharp* and S. Oross III. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

Alzheimer's patients exhibit visual losses including elevated contrast sensitivity thresholds, blue-yellow color deficiencies, and elevated thresholds for detecting coherent motion in kinematosgrams. To assess the degree to which profiles of visual losses may differentiate between clinical populations, we have begun comparing the performance of Alzheimer's patients to three groups of adults with mental retardation: unspecified etiology, young (<35 years) Down Syndrome, and old (>35 years) Down syndrome. Measurements of visual acuity, contrast sensitivity, color perception, and detection of motion coherence were obtained. Detection of motion coherence was assessed using a 2AFC task in which subjects had to determine which of two simultaneously presented kinematosgrams contained coherent motion (signal strength varied between 1 and 100%). The other kinematosgram contained only random noise. Rules to standards of each group were comparable with control subjects, all groups exhibited elevated visual acuity, contrast sensitivity and motion coherence detection thresholds. Variations in these threshold elevations suggest that profiles of visual losses may differentiate between the groups. Discussion concerning the construction and use of a battery of visual tasks for assessing the neurological status of various clinical populations will be advanced.

Supported by HD29556

**PROCESSING PROFILES AT THE RETINAL VERTICAL MIDLINE OF A CALLOSOTOMY PATIENT.** R. Fendrich*, C. Westinger, MS Gazzana. Center for Neuroscience, UC, Davis 95616.

Previously, we reported that a calllosotomy patient could not compare small outline shapes presented 15° from the retinal vertical midline with comparison shapes in the opposing visual field. This foveal splitting paradigm argued against the attribution of macular sparing to a zone of nonspatial overlap. We now report evidence that a narrow zone of overlap does occur in the human retinal vertical midline, but is limited in its ability to convey visual information to the cerebral hemisphere contralateral to each hemiretina. Pairs of 2° x 2° square waves of contrast were flashed alternately from each eye to each hemisphere. The subject judged which gratings had the same orientation. Retinal stabilization insured the grating patches remained properly stabilized. When the gratings were presented for 200 msec or their medial edges were 2° from the retinal vertical midline, the subject performed as well near the center. When presentations lasted 2 seconds and the grating medial edges were 1° from the midline, above chance performance was maintained with the 1°, 2°, and 4-cpd gratings. Performance degraded when both gratings were displaced 2° upward from the horizontal meridian, but was unaffected or improved by a like downward displacement. Remarkably, accuracy rates improved when the gratings were offset vertically away from each other (one 2° up, one 2° down). Supported by NIH/NINDS PO1 NS17778-12 A PHS NS1443-01


Two patients, both with right hemispherectomy, one with right-sided removal of the temporo-parietal-occipital cortex and four normal control subjects were tested for spatial summation in the visual field across the vertical meridian. The double stimulus paradigm described by Tassini et al (1984) and Marzi et al (1986) was used. Monocular eye fixation was monitored with a pupil/orbital reflection tracking system and all subjects used their right eye. Single or pairs of flashes (LEDs) were randomly presented during 10 msec at 10° or 30° to the left or to the right of the fixation point, either within or across hemispheres. Subjects had to respond as fast as possible to the stimuli by a keypress. As expected, control subjects reacted more quickly to double stimuli than to single ones. Although the patients never responded to stimuli in the blind field, they were significantly faster when two flashes were displayed in both hemifield rather than when a single flash was presented in the intact hemifield only. This summation effect across hemifields in subjects in whom a whole cerebral hemisphere has been removed or deafferented supports the possibility that alternate visual structures (e.g. superior colliculi) contribute to residual vision in the blind field. Supported by NSERC OGNEN 012.

**Residual Vision in The Blind Field after Partial or Complete Hemispherectomy.** C.M. Weisberger1*, R. Fendrich1, A. Pittet2 and M.S. Gazzana.*

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2Montreal Neurological Institute, Montreal H3A 2B4, Canada.

One patient with left functional hemispherectomy (JB) and one with right-sided removal of the temporo-parietal-occipital cortex (SE) were tested for residual vision in their blind field. Using image stabilization procedures, we identified a zone of residual vision along the vertical meridian in each patient. The lateral edge of this zone was generally within 3.5° of the vertical meridian, although in both patients we found local area of detection beyond 3.5°. The region of residual vision was not identical in the two patients. In JB it was confined to the superior quadrant; in SE it was present in both superior and inferior quadrants. Each patient could detect stimuli and correctly perform shape discriminations within the zone of residual vision, but not outside of it. In contrast, neither patient was able to name complex line drawings presented in the zone.

Acuity profile assessments in each patient’s seeing field argue against the attribution of these abilities to eccentric fixation. Given the reported complete absence or deafferentation of a hemisphere contralateral to the residual vision, alternate structures would be mediating this residual vision. The superior colliculi and/or the remaining cerebral hemisphere could be candidate structures.

Supported by NIH/NINDS PO1 NS17778-12, the McDonnell-Pew Foundation and NSERC OGNEN 012.
FURTHER RESULTS FROM A TRAINING OF BRIGHTNESS PERCEPTION AND REACTION TIMES IN HOMONYMUS HEMIANDROPA. L. Dornheim, F. Schneiuel*. Inst. for Medical Psychology, Med. Univ. of Luebeck, Luebeck Germany, GER 23538.

Visual field (VF) defects in patients with lesions of the post- chiasmatic visual system demonstrate a reduction of perceived brightness at the border of the residual VF. Dornheim and Schneiuel (1994) showed that visual functions can be improved by a rehabilitation training using a magnitude estimation technique of apparent brightness. Now further results are demonstrated for a female patient (age 73 yrs.) who suffered from an incomplete homonymous hemianopia of the right VF caused by stroke. Monocular scaling of threshold and super threshold stimuli and a specific training of light-dark discrimination was performed by a computerized perimeter within the lower right quadrant caused a VF enlargement, a reduction of reaction times and threshold as well as an increased of perceived brightness within the trained area and an increase of visual acuity. The authors explain the improvements by recovery of visual cortex neurons due to repetitive stimulation during selective attention.

DYSPHONEDIETIC DYSLEXICS DEMONSTRATE A MAGNOCELLULAR PATHWAY DEFECT. W.H. Ridder, III. E. Borsting, M. Cooper, B. McNeel, and P. Simmons*. Southern California College of Optometry, Fullerton, CA., 92631.

A review of the literature reveals that approximately 75% of dyslexics have a processing deficit in the magnocellular pathway (Lovegrove et al., 1990). In addition, a recent report demonstrated that the dyslexic subject, who comprises 10 to 30% of the dyslexic population, does not have an abnormal magnocellular pathway (Ridder et al., 1995). These results suggest that the other two subtypes of dyslexia (dysphonodiabetic and dysphonediabetic) should have a magnocellular pathway defect. The purpose of this study was to determine if dysphonodiabetic dyslexics exhibit a magnocellular pathway defect. Four dysphonodiabetic dyslexics and five age and sex matched, normal controls were examined. All subjects had normal intelligence and educational opportunities, no ocular disease, sensory impairments, or systemic pathology. The presence of dyslexia was determined with the Adult Dyslexia Test. Contrast sensitivity functions were determined with vertically oriented sine wave gratings (0.5, 1.0, 2.0, 4.0, 8.0, and 12.0 deg. in frequency defining at 0.1 and 10.0 Hz) and for a full field flickering stimulus (5, 10, 15, 20 and 25 Hz) by employing the temporal, 2 alternative, forced-choice technique. Unpaired t-tests demonstrated significant differences existed between the dysphonodiabetic dyslexics and the normal controls for the data obtained at low spatial and high temporal frequencies. These results suggest that dysphonodiabetic dyslexics have a magnocellular pathway defect.


We report here evidence to suggest visual sensitivity in normal subjects can vary as a function of eccentricity. In a block of trials, subjects were asked to judge the width of the objects by stating which of five different blocks (numbered 1 to 5) it was. In addition of trials, subjects indicated how wide they thought the block was by opening their forefinger and thumb to the width perceived. In addition to these two explicit judgements of object width, another more implicit judgement was obtained by simply requiring subjects to reach out and grasp the object using a precision grip (with forefinger and thumb). Based on previous work, it was expected that grip aperture would be strongly correlated with object width. In this case and in the previous manual estimation condition, the opening between the forefinger and thumb was measured using standard opto-electronic recording (WATSMART). The effect of retinal eccentricity on the object estimation varied as a function of the response used. When verbal responses were required, size estimates decreased as a function of retinal eccentricity; when manual estimates were required, grip aperture was reduced as required to grasp the object, their grip aperture increased with eccentricity. In short, increasing retinal eccentricity had different effects on judgements of object width, and object estimation. This further support to the proposal that visual perception and the visual control of action depend on different transformations of visual information (Goodale, Curr.Opin Neurobiol 3: 1993). This research was supported by a grant from MRCC to M.A.
VISUAL REPRESENTATIONS FOR CLASSIFYING 3D OBJECTS.
Hiroshi Ando* and Satoshi Suzuki,
ATR Human Information Processing Research Labs., Kyoto, Japan.

There have been increasing interests in how the brain represents visual objects and object recognition. Physiological experiments have shown that IT neurons may represent local features or prototypes of learned objects (Shoemake et al., Nature 360, 943, 1992; Lopes et al., Neurosci. Abstr., 1993). Psychophysical studies have indicated that the visual system may extract view-specific prototypes of 3D objects in identification tasks where human subjects have to recognize the target object from distractors (Edelman & Bülthoff, Vision Research, 32, 2385, 1992). This research provides a physical and computational perspective to investigate whether the visual system may extract global prototypes or local distinctive information in 3D object classification tasks.

In the present study, we employed a 2D views of a fixed set of wire-frame 3D objects. The results show that some views yield higher error rates. To investigate what representations are extracted, we examined three network models, i.e., the MLP (multi-layer perceptron) network which can extract local linear features, the GRBF (generalized radial basis function) network which extracts global prototypes, and an extension of HBF (hyper basis function) network that allows to emphasize various local templates. The simulations on the same objects used for the psychophysics showed that with a small number of hidden units the MLP and HBF networks perform better than the GRBF network. The results suggest that it is useful to extract local distinctive information that is stable over different view directions, such as acute angles formed by successive segments. Analyses of errors reveal that some subjects made errors at particular views similar to the views these networks fail, suggesting that the visual system may extract local distinctive information for classifying 3D objects.

MAGNETIC AND ELECTRIC OSCILLATORY RESPONSES TO FLASH SIMULATION EFFECTS OF LUMINANCE AND ECCENTRICITY. L. Lopes*1 M. Peresson*1 A. Pascualar*1 G.L. Romani1 W.G. Barnard4 1TABB, University of Chieti, Italy, 2ESS-CNRE, Rome Italy, 3DISM, Center for Cerebral Neurophysiology University of Genova, Italy, 4Dept. Psychol. SUNY, Stony Brook, NY. In this study we wanted to further characterize the origin and possible functional role of these responses.

We measured electric and magnetic responses to full-field and to various spots that progressively varied in area and eccentricity from point of fixation (5° to 30°), alternatively in the upper left and right quadrants of the visual field. Eight healthy volunteers were used. Simulation was delivered monocularly, to the left eye with a Glass FS82 (10 s duration, 0.5 Hz, 3.5 cd/m² at full-field) 90 dB masking white noise) The stimuli could be delivered without moving the subject. One position, covering an area of 250 mm² over the left occipital lobe, was chosen as a stable magnetic recording.

Electric and magnetic oscillatory responses during full-field stimulation, were obtained. When changing the stimulus to eccentric and smaller spots, the retinal oscillatory potentials tended to disappear, together with ERG, while the cortical oscillatory responses could still be detected with features that varied as a function of eccentricity and luminance. Such findings would indicate that the mechanisms underlying the generation of oscillatory responses in the cortex are partly independent of the retinal oscillatory potentials and that a magnification occurs in the visual system.


It is known that acute and semi-acute hypobaric conditions reduce the (relative) light, which lasts for at least 3 days. To what extent red/green color vision is affected under acute normobaric hypoxia and hypoxia, hyperbaric conditions, and chronically increasing hypobaric hypoxia has not been reported before. The red-green balance-flicker (16 Hz) fusion point was measured psychophysically for normal subjects with the portable OSCAR color vision tester. The red/green (R/G) modulation sensitivity ratio is enhanced by normocapnic acute hypoxia (10-13 kPa O2, 0-11% increased SaO2, 58-83%) and reduced by normocapnic hypoxia (60-100 kPa O2, 2-10% decrease, 6 subjects). The effects are maximal after 20 min of exposure. Excretion (>30 min 150 W) also results in an enhanced (2-9%) R/G ratio. Hyperbaric air pressures, examined up to 520 kPa, reduce less less. An hypothetical explanation of this lesser reduction is an opposite effect of N2 narcosis (and hypercarbia) at pressures ≥300 kPa. Above 100 kPa O2 the effect of O2 saturates (5 subjects).

This may partly be due to hypercapnia since normocapnic CO2 mixtures (2 and 4 kPa) resulted in an increased sensitivity ratio (2 subjects).

3D COLOR SENSATIONS IN HONEYBEES? Werner Backhaus*, Institute for Neurobiology, C/o Institut für Biophysics, Friedrich-Alexander-Universität Erlangen-Nürnberg, 91058 Erlangen, Germany.

Neuronal color coding and color choice behavior of honeybees is very well described by the color theory for the bee (Backhaus, 1991, Vis. Res. 31, 1381; 1992, 32, 1425). The results of ordinary color training experiments are described by the color theory on the basis of a dual neurone mechanism with two color opponent coding neurons. The results of double color training experiments, in which two color stimuli were trained simultaneously cannot be explained by a model on the basis of the electrical properties of neurons. The choices rather appear to be related to color sensations (Backhaus & Kratzsch, 1993, Proc. 21st Symp. Science of Color, 30-35; Color Res. and Thiene).

A quantitative model is presented which describes color sensations in bees to consist of unique-colors, as in humans (Herings, 1905, Leipzig). Since bees ignore brightness differences in color training experiments, only five unique colors are needed. The amounts of unique-colors are assumed to be linearly related to the electrical excitation values of the two color opponent coding neurons. In double color training experiments, honeybees appear to distinguish one of the color-object colors which the two trained colors have in common and to choose the stimulus in the tests according to the amounts of these unique-colors. Quantitative predictions for further experiments are derived from the model for further testing the hypothesis about the existence of unique-color sensations in the honeybee.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: REFLEX FUNCTION I

A STUDY OF CUTANEOUS INHIBITORY INPUT TO A SPINAL NOCICEPTIVE MOTOR SYSTEM. B.B. Naud* and J. Schoenmaker. Department of Physiology and Biophysics, University of Lund, Slottsvagen 15, S-22362, Lund, Sweden.

The nociceptive withdrawal reflex system has been extensively studied from both motor and sensory aspects. A 'modulatory system' of this system has been recently described, each 'module' acting on a single or a few sympathetic muscles (Schoenmaker et al. News in Physiological Sciences 3, in press). Each module receives excitatory input from the skin area withdrawn by its effects (Weeden, 1992). We have now extended the cutaneous inhibitory input system to this motor system in the decerebrate spinal cat (Wankels, 1988).

Reflex responses in single hindlimb muscles evoked by sustained noxious pinch (2N, 1mm²) were recorded using extracellular recording techniques. Inhibitory receptive fields of mm.permenosus longus and brevis, extensor digitorum longus, tibialis anterior and biceps posteriores were mapped using mechanical pinch (up to 2.5N, 1 mm² and Dye-laser thermal stimulation (50-300 mJ, 1 mm diameter). For each muscle, the reflex responses could be inhibited by electrical stimulation (5-10 Hz, 1 ms) of the skin area which is moved towards the cutaneous stimulation on the muscle. Noxious stimulus produced an inhibitory effect in the reflex elicited by cutaneous stimulii, indicating a nociceptive inhibitory input. In intact animals the excitatory and inhibitory receptive fields of withdrawal reflexes to single muscles reflects the movement pattern caused by the muscles. Both the excitatory and inhibitory actions on this system protect the body from injury.

ESTIMATED NET SYNAPTIC POTENTIAL IN HUMAN MOTORNEURONES. K.S. Turner* and H.B. Cheng, Department of Physiology, University of Adelaide, S.A, 5005, Australia.

Although recording single motor unit activity from human muscles is technically simple, quantifying reflex responses has not been easy. The most commonly used method for quantifying the reflex response has been the peristimulus time histogram (PSTH) and its cumulative sum (CUSUM) which illustrates the change in the firing probability of the single motor unit in response to a stimulus. According to this method, an increase in the firing probability relative to the pre-stimulus period indicates excitation and a reduction represents inhibition. However, it has been demonstrated that the stimulus may induce synchronous activity of the unit and this may induce several peaks in the histogram many of which are not due to the stimulus-induced extra activity in the motoneurone. These peaks are, in fact, due to the summation of the background activity from a fixed point of time "the autocorrelation effect". The frequency of firing of motor units, however, avoids these synchronous activity-induced false peaks and troughs. Furthermore, the frequency of firing of a unique-colors which the two trained colors have in common and to choose the stimulus in the tests according to the amounts of these unique-colors. Quantitative predictions for further experiments are derived from the model for further testing the hypothesis about the existence of unique-color sensations in the honeybee.
REFLEX RESPONSES TO LOW-INTENSITY STIMULATION OF THE SURAL, TIBIAL, AND PERONEAL NERVES DURING HUMAN WALKING. B.M.H. Yan West, P.A.M. Oosterhoff and J. Doymas*. Dept of Medical Physics & Biophysics, University of Nijmegen, 6500 HW Nijmegen, Netherlands. The skin of the foot is innervated by 3 nerves, namely the sural, tibial, and peroneal nerve. Most studies on the reflex responses to stimulation of these nerves during human locomotion have concentrated on only 2 of these nerves. The question arises whether the reflexes, and their phase-dependent modulation in locomotion, show differences for these different nerves. The purpose of the present study was to compare the reflex responses to stimulation of these 3 nerves in the same subjects, while walking on a treadmill.

Both facilitatory and suppressive reflex responses with a latency of about 40 ms were observed following a perception threshold of 3.5 ms for all 3 nerves. In general, the responses for the 3 nerves are of the same order of magnitude. The pattern of the responses can be different, however. For example, the responses in bisected femorotibial nerve were generally facilitatory during the whole step cycle, whereas responses in IF to tibial nerve stimulation at end swing were often suppressive. In tibialis anterior suppression at end swing were observed in the response pattern for all 3 nerves. During the stance phase, however, a clear difference was found: generally no responses were found for sural and peroneal nerve stimulation, but large facilitatory responses were often found during mid to end stance for tibial nerve stimulation.

In conclusion, the low-threshold afferents from the 3 nerves, innervating the skin of the foot, have both excitatory and inhibitory pathways to various leg muscles. The phase-dependent reflex modulation can be different, presumably by adjusting the balance of activity in these opposite paths. This suggests the existence of differences in the reflex pathways for the different nerves, which can be controlled separately during the course of the step cycle.

648.3

DOES THE NERVE BRANCH PATTERN REFLECT A RE-MODELING WITHIN THE MOTONEURON POOL - MUSCLE COMPLEX OF OLDER RATS? S. Vanden Noven*, H.K. Lietum and M.J. Partidos. Sch. of Physical & Occupational Theraphy, McGill Univ., Montreal, QC H3G 1Y5. In this laboratory, the medial gastrocnemius (MG) motoneuron pool was studied in young rats (Sprague-Dawley; Fischer 344) being used in various morphological and electrophysiological studies in order to further our understanding of the mechanisms underlying the function of the motoneuron pool. The two extra-muscular nerve branches innervating the MG muscle in the Sprague-Dawley rat subserve a physiological and histochmical compartmentalization (Vanden Noven et al., 1994). The afferent contributions of these two muscle nerve branch patterns were also been found to differ between rats of different ages (Vanden Noven, 1993). In this study, dorsal root volleys (Group I stimulus strength) and the MG muscle nerve branch pattern was compared between young adult (n=5) and older animals (12-23 mo; n=5). Neural potentials, evoked by stimulation of the L4-L5 dorsal roots, were recorded from each of the two nerve branches. The MG muscle nerve branch pattern was more complex and the topographic distribution of DR afferents entering the spinal cord was disrupted in the older age group. Taken together with other data on motoneuron and muscle properties, results suggest that the MG complex undergoes a re-organization with age.

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648.5

EFFECTS OF THE SEROTONIN-2 AGONIST DOI ON S VS. F MOTONEURONS IN VIVO. D.H. Lee, J.F. Miller, W.Z. Rymer and C.J. Heckman*. Physiology, Northwestern Univ. Med. Sch. and VA, Lakeside Hospital, Chicago, IL 60611. The orderly recruitment of motor units is largely due to systematic differences in the threshold currents (theobases) of slow twitches (S) vs. fast twitches (F) motoneurons (MNs). Serotonergic (5HT) agonists can greatly reduce theobases and our goal was to investigate the relative amount of this decrease in theobase in S vs. F MNs.

DOI, a 5HT-2 agonist, has long lasting actions in vivo (>2 hrs; see the companion poster by Miller et al.). The intrinsic properties of two populations of triceps surae MNs in the decerebrate cat were compared in control conditions and following 15 mg/kg administration of DOI (0.5-1 mg/kg). The preparation was spinalized at T10 to eliminate the effects of descending SHI tracts.

The average decrease of the population (8.5 mA; range: 6 to 21 mA; n=6) was significantly less (t-test, p<0.005) than that of the control population (12.6 mA; range: 8 to 32 mA; n=4). DOI MNs with negative rheobase (n=12) had sustained rhythmic firing in the absence of any injected current. Negative rheobase MNs had slow conduction velocities (CVs) and were probably type S. However, the slopes of the regression lines for the rheobase-CV relations were not significantly different in the two populations, indicating that the effect of DOI was approximately the same in both S and F MNs. These data suggest that DOI input can enhance the excitability of the motoneuron pool without altering its recruitment order.

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648.6

EFFECTS OF THE SEROTONIN AGONIST DOI ON FORCES AND STRICTION REFLEXES OF THE DECEREBRATED CAT. J.F. Miller*, K.D. Paul, W.Z. Rymer and C.J. Heckman. Physiology, Northwestern Univ. Med. Sch. and VA, Lakeside Hospital, Chicago IL 60611. 5-HT2 agonists produce substantial depolarizations of spinal motoneurons. Such actions should substantially increase the input-output gain of the motoneuron pool. We therefore investigated the effect of the DOI 2A/2C agonist on the reflex output of the triceps surae muscles in the decerebrate cat preparation.

The stretch reflex and background force of extensor MNs are greatly enhanced in the decerebrate cat. Intracerebral administration of DOI (1-5 μg/ml; cumulative) further increased stretch reflex amplitude by 30-50%. Even more striking changes in background force were observed. For example, soleus background force typically increased 2-4 fold after DOI, and in one experiment exceeded 20 N, which is near the maximum force of the intact cat. Similar results were seen following i.v. administration of DOI (0.5-1 mg/kg).

The duration of DOI's actions by either route typically exceeded 120 min. Ketorolac (0.5-1 mg/kg i.v.) transiently reversed the effects of DOI for 30-45 min. In one experiment, the spinal cord was transected at T10, resulting in a great reduction in background force and stretch reflex amplitude. DOI (1 mg/kg i.v) restored force and reflexes to their pre-spinalized level. These results show that serotonergic synaptic input can greatly enhance the output of the motoneuron pool.

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648.7

DISYNAPTIC AUTOGENIC AND HETEROGENIC EXCITATION FROM GROUP I AFFERENTS IN HINDLIMB EXTENSOR MOTONEURONS DURING FICTIVE LOCOMOTION. M.J. Angel*, P. Guerret, J. Jimenez* and D.A. McCrea. Dept of Physiology, U. of Manitoba, Winnipeg, CANADA, R3E 3W1 and ITA-MX. Mexico.

Stimulation of plantar group I afferents during the extensor phase of fictive locomotion evokes disynaptic EPSPs in medial gastrocnemius motoneurons, thus replacing the inhibitory potentials evoked at rest (McCrea et al., Congr. on Physiol. Sci. 1993). In the present study, we examined the distribution of disynaptic EPSPs from ankle extensor group I afferents to hindlimb extensor motoneurons during MLR-evoked fictive locomotion by extending the stimulus and noting the extensor nerves (single shocks, 1.5 milliseconds threshold) produced EPSPs in ankle and hip extensor motoneurons and in the bifunctional PBS motoneurons (hip extensor + knee flexor). Autogenetic excitation was also observed in ankle and hip extensor motoneurons during extension. The latencies of the EPSPs (1.1-1.9 ms) suggest the opening of a disynaptic pathway from group I afferents to extensors during extension. The presence of group I autogenic and heterogenic, i.e., disynaptic excitation is a functional reorganization of ankle extensor group I reflexes during locomotion. The switch from inhibition at rest to excitation during locomotion indicates recruitment of a previously undisclosed population of excitatory group I interneurons. Activation of these interneurons by group I afferents during extension enhances hindlimb extensor motoneurons and acts as a positive feedback system enhancing centrally programmed extension.

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BOTH DISYNAPTIC AND LONGER LATENCY INTERNEURONAL PATHWAYS MEDIATE EXTENSION ENHANCEMENT EVOKED BY GROUP I MUSCLE AFFERENTS DURING FICTIVE LOCOMOTION. P. Guerret*, M.J. Angel, J. Jimenez* and D.A. McCrea. Dept of Physiology, U. of Manitoba, Canada and ITA-MX.

Trains of group I stimuli to ankle extensor nerves increase the amplitude and duration of extension electromyogram locomotor bursts (Guerret et al. Soc. Neurosci. 19:61-15, 1993). The present study examined the synaptic effects of group I stimulation in the intact and in spinalized animals. The group I-evoked fictive locomotion. Plantar nerve stimulation (1.5 times threshold; 20 pulses; 200Hz) during extension increased the amplitude and duration of hindlimb extensor motoneuron locomotor drive (LDPs). The increase in LDP amplitude began within 10 ms after the first shock in the train and often continued well beyond the duration of the stimulus train. Accompanying the LDP enhancement, and depending on the preparation, were distinct disynaptic excitatory or post-synaptic potentials following each stimulus pulse. In most cases, the depolarization produced by the LDP enhancement was larger than the disynaptic depolarizations. Since LDP enhancement occurred in the absence of a coincident muscle fiber action potential, the pathway for the group I afferents is part of a positive feedback system during locomotion that shapes the amplitude and duration of centrally programmed extension bursts.

Supported by the MRC and the Rick Hansen Legacy Fund.

Group I afferents from extensor muscles exhibit a reflex reversal during locomotion that leads to excitation of the extensor motoneuronal pool. This excitation can be prevented by transcutaneous stimulation of the extensors during fictive locomotion. A question that arises is whether this excitatory actions are functionally relevant in a moving animal that is receiving phasic afferent input from many sources.

To address this issue, extensor nerves in the hindlimbs of decerebrate cats were stimulated, while the animals walked spontaneously on a treadmill. The ankle (L4-S1 L & P) and knee (L6 & V) extensor nerves were dissected, cut, and enclosed in stimulation cuffs. Stimulation of each nerve with trains (400-1500 ms, 200 Hz, 1.8 X T) during the extensor portion of the step cycle resulted in a prolongation of the extensor burst. Flexor burst delay was initiated by delayed stimulation but not always to the end of the stimulus volley. Stimulation during early flexion resulted in premature termination of the flexor burst and a delayed excitation of the extensors, followed by a reinflation of flexion coincident with stimulus offset. When the stimulation intensity was reduced to sub threshold levels for each nerve, it was possible to stimulate two nerves simultaneously and elicit an increase in the extensor duration. This nonlinear summation suggests that the regulation of the stance phase be partly regulated by group I afferents from many extensor muscles converging onto a common interneuronal pool.

Since the observed effects are similar to those attributed to group II afferents in reduced preparations, it is concluded that a reduction in force feedback to lb extensor afferents is necessary for the initiation of the swing phase in a walking animal.


Recently, it has been shown that autogenic inhibition arising from group II afferents in extensor muscles is largely eliminated during walking in reduced cut preparation (Conway et al. 1987 Expt Brain Res 68:643, Pearson & Collins 1993 J Neurophysiol 70:1009).

Instead, a long lasting autogenic excitation is seen. The purpose of this study was to determine whether this change is also apparent in humans. Putative Ib responses can be elicited by conditioning the soleus H-reflex with a stimulus to the medial gastrocnemius nerve at or just below below threshold (e.g., Pieper & Deuschl, J 1979 Brain Res 166:176). The response was observed both in quiet sitting and in the stance phase of walking. The inhibition during sitting, observed by others, was reproduced. Preliminary results indicate this inhibition was either reduced or eliminated in walking. Sometimes, this was replaced by excitation. Hence, qualitatively, the results support those obtained from the cat, but quantitatively, the effect was considerably more modest in the normal human.

This work was supported by MRC to JFY and scholarships from NSERC and AHFMF to MJS.

THE EFFECTS OF A TONIC INCREASE OF PRESYNAPTIC INHIBITION OF MUSCLE SPINDLE AFFERENTS ON THE STRETCH REFLEX PARAMETERS OF THE CAT. C. Capaday, C. Centre de Recherche en Neurobiologie, Universite Laval, Quebec City, Quebec, Canada, G1K 7P4.

Experiments were done in cats decerebrated at the pontocerebellar rectumotomy or by bilateral pontine lesions to determine how a tonic increase of presynaptic inhibition of the intraspinous terminals of muscle spindle afferents affected the slow component of the soleus stretch reflex (s/r). Baclofen, a specific GABA receptor agonist was injected into the brains of the cats in the dose of 1 mg/kg. The slow component of the stretch reflex was reduced by baclofen as a function of the background force level was determined by stretching the muscle with a square pulse of 2 mm and 500 ms duration. The Baclofen dose-dependently reduced the intra-synaptic component of the stretch reflex, without any effect on the extra-synaptic component. Baclofen-induced reduction of the stretch reflex was most evident in the dose of 1 mg/kg. Baclofen-induced reduction of the stretch reflex was most evident in the dose of 1 mg/kg. The threshold of the stimulation used required to elicit the stretch reflex was not affected by baclofen. The results suggest that the tonic increase of presynaptic inhibition of the intraspinous terminals of muscle afferents. However, the present data suggest that the tonic increase of presynaptic inhibition of the intraspinous terminals of muscle afferents has no effect on the slow component of the stretch reflex and the threshold of the stimulation used required to elicit the stretch reflex was not affected by baclofen.

In conclusion, these data suggest that the tonic increase of presynaptic inhibition of the intraspinous terminals of muscle afferents has no effect on the slow component of the stretch reflex and the threshold of the stimulation used required to elicit the stretch reflex was not affected by baclofen.
The innervation of the dorsal and ventral horns of the rat spinal cord by axons descending from the locus coeruleus/subcoeruleus complex and A5 cell group. M. A. Sant'Ana, E. Polgar, and A. Z. Ribeiro. Department of Anatomy, University Medical School, Dobreson, H-4014 Hungary.

The spinal projections of noradrenergic brainstem nuclei that influence complex sensory and motor activities in the spinal cord were studied in a rat by using the Phascolosaurus vulgaris leucocephalus (PHA-L). After injecting PHA-L unilaterally into the nucleus coeruleus, nucleus subcoeruleus, and A5 cell group, labelled fibre terminals and terminals were detected in the spinal cord, from which spinal cord nuclei, spinothalamic, and cutaneous segments of the spinal cord. Most of the terminals (60-80%) from all three nuclei were found in the ventral horn laminae VII-VIII. The superficial dorsal horn (lamina I-II) received a relatively sparse innervation from the locus coeruleus and A5 cell group. The subcoeruleus complex, however, projected more dorsally to the dorsal horn. More than 30% of the terminals arising from the subcoeruleus complex were revealed in lamina I-II at the level of thoracic and lumbar segments on both sides of the spinal cord.

To study the postynaptic targets of these descending fibre sections were stained for both PHA-L and calbindin-D28k (CaB), a calcium-binding protein that have been reported to be markers of certain subsets of spinal neurons stained including stacked cells and supraspinally projecting neurons in the dorsal and Remilows-cell in the ventral horn. All of the aforementioned terminals of CaB-immunoreactive fibres were stained to receive contacts from fibers descending from the investigated noradrenergic brainstem nuclei. Synaptic contacts of terminals in laminae I-II and laminae VII-VIII as well as GABA and glycine immunoreactivities of their postynaptic targets were also investigated in a correlative electron microscopic study.

Deep periaqueductal gray neurons project to the spinal cord in the cat. L. J. Mouton, L. Kerstens, and G. Holstege. Dept. Anatomy and Embryology, Faculty of Medicine, Rijksuniversiteit Gent, the Netherlands.

The mesencephalic periaqueductal gray (PAG) plays an important role in many different functions, such as the regulation of pain, cardiovascular control, vocalization, and motor coordination. In the present tracing study in the cat (Mouton and Holstege, 1994) HRP injections in the upper thamic and cervical spinal cord resulted in many strongly labeled neurons in the ventrolateral PAG, but also a few faintly labeled small neurons were found at the level of the dorsal PAG and the adjacent intercollicular zone. Nothing was found for the function of these small dorsal border PAG-spinal neurons. The purpose of the present retrograde and anterograde labeling study was to exactly describe this dorsal border PAG-spinal pathway.

In nine cats WGA-HRP was injected in the spinal cord, each at a different spinal level. Prior to the injection a hemisection was made rostral to the injection site. In order to identify and count the retrogradely labeled PAG neurons every 40 μm transverse section of the brainstem was incubated using the TMB method. In an anterograde tracing study WGA-HRP injections were made in the area which contained the small dorsal PAG-spinal neurons.

Results show that there exist several hundreds of relatively small neurons at the dorsal border of the substantrial PAG projecting to the cervical and thoracic spinal cord. Only a few dorsal border PAG neurons seem to project to the lower lumbar cord, while almost one hundred neurons project to the cervical spinal cord. The neurons project ipsilaterally to the area immediately surrounding the central canal, but not corresponding to laminas X. Further electronmicroscopic studies are needed to more precisely reveal the nature of this pathway.

The deep mesencephalic nucleus (DM) is a deep extensive territory included in the mesencephalic reticular formation. In 32 rats small deposits of Phascolosaurus vulgaris leucocephalus (PHA-L) were placed in the DM. Staining different sections of different DM regions resulted in the thalamus, zona incerta, superior colliculus, accessory oculomotor nuclei, peri-aqueductal gray substance, reticular formation, periaquedural, and cranial nuclei. In the rostral and central lateral thalamic nuclei, ventrolateral geniculate, intergeniculate, and subgeniculate nuclei, zona incerta, anterior pretectal nucleus and superior colliculus (superficial layers) are targeted mainly by ventral PAG, DPG, and the lobar thalamic nuclei, ventrolateral geniculate, intergeniculate, and subgeniculate nuclei, zona incerta, anterior pretectal nucleus and superior colliculus (superficial layers) are targeted mainly by ventral PAG, DPG, and the lobar thalamic nuclei, ventrolateral geniculate, intergeniculate, and subgeniculate nuclei of the brainstem were incubated using tetramethylrhodamine-dextran. The resulting light labelling in the dorso-lateral thalamic segments was cut, and the results showed that the terminals from the deep mixed-light and electronmicroscopic study were obtained to directly visualize the projections from the NMR to the lumbar spinal cord. Anterogradely labeled PAG-spinal neurons, in four cases injections of wheat germ agglutinin horseradish peroxidase (WGA-HRP) were made in the NMR. In two of these cases plain HRP was injected in hamster nuclei to retrogradely label PAG-neurones receiving projections from the NMR. In all cases the labelling was studied using the TMB method.

The results show that NMR terminals form asymmetrical contacts on the lateral thalamic nuclei, zona incerta, and the periaqueductal gray substance. The deep mesencephalic nucleus (DM) is a deep extensive territory included in the mesencephalic reticular formation. In 32 rats small deposits of Phascolosaurus vulgaris leucocephalus (PHA-L) were placed in the DM. Staining different sections of different DM regions resulted in the thalamus, zona incerta, superior colliculus, accessory oculomotor nuclei, peri-aqueductal gray substance, reticular formation, periaquedural, and cranial nuclei. In the rostral and central lateral thalamic nuclei, ventrolateral geniculate, intergeniculate, and subgeniculate nuclei, zona incerta, anterior pretectal nucleus and superior colliculus (superficial layers) are targeted mainly by ventral PAG, DPG, and the lobar thalamic nuclei, ventrolateral geniculate, intergeniculate, and subgeniculate nuclei of the brainstem were incubated using tetramethylrhodamine-dextran. The resulting light labelling in the dorso-lateral thalamic segments was cut, and the results showed that the terminals from the deep mixed-light and electronmicroscopic study were obtained to directly visualize the projections from the NMR to the lumbar spinal cord. Anterogradely labeled PAG-spinal neurons, in four cases injections of wheat germ agglutinin horseradish peroxidase (WGA-HRP) were made in the NMR. In two of these cases plain HRP was injected in hamster nuclei to retrogradely label PAG-neurones receiving projections from the NMR. In all cases the labelling was studied using the TMB method.

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469.7


A centre de recherche en sciences neurologiques, Laval, Quebec, Canada, and the Montreal Neurological Institute, Montreal, Canada. (H/C 337, Department of Surgery, University of Florida, Gainesville, Department of Neurology, University of Quebec à Montreal.)

It has been shown that effective neurons supplying the muscular and daceletic muscles of the rabbit are located in two brain stem nuclei: the trigeminal motor nucleus (ncl V) and the spinal motor nucleus (ncl VII). In the present study, following hemisection in pentobarbital sodium anesthesia. We have been able to conduct two series of experiments to characterize the muscular effective neurons of group-K. In the first, retrograde labelling with fluorescent dyes (together and fastblue) was used. The nuclei were excised, the central parts of the brain stem and neuron were killed after 8 days. We found that the nerve branches to the specific set of muscles of the calvaria. All of the muscle areas are all represented in group-K, that the effective nerves to those branches are interconnected. As a first step towards understanding the function of group-K neurons, their immunoreactivity to choline-synthesizing enzyme (ACCh), and the specific marker for cholinergic neurons was tested. Absence, immunostaining and perfusion. After fixation, vibratome coronal sections of the brain tissue were cut at 30μm. For sections were treated with a set of immunohistochemical dye (Code ACH), using the avidin-biotin peroxidase method. A reaction of the majority of cells in group-K were immunostained and were of the same size and shape as retrogradely labelled neurons. This suggests that neurons of group-K are cholinergic and are likely to be monoaminergic. However, group-K neurons are fusiform and smaller in size (mean diameter=31μm) than ncl V cells, which are round or oval (mean diameter=44μm). This suggests that the group-K neurons supplying the muscular muscles could be ganglia motoneurons, but this is contradicted by the fact that the daceletic muscle, which contains no muscle spindles in the rabbit, is also innervated by this nucleus.

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469.9


A light microscope study of the dorsal and ventral roots at the lumbar-sacral junction of the spinal cord was made in the opossum in order to establish the total number of myelinated fibres and their size distribution. Dorsal and ventral roots were removed from deeply anaesthetized animals and were immersed overnight in 2% glutaraldehyde-2.5% paraformaldehyde in cacodylate buffer (pH 7.3). The tissue was rinsed, postfixed for 4 hours with 1% osmium, rinsed again, dehydrated, embedded in ethanol and embedded in Spurr's medium. Sections (1μm) were cut from the proximal part of the spinal cord, stained with toluidine blue and dried. They were photographed and used as a basis for obtaining a montage of the whole root section. An average of 890 ventral root fibres are myelinated. They can be divided in two populations: small fibres having a mean diameter of 8±2μm (42%), and large fibres having a mean diameter of 18±4μm (58%). myelin sheaths included. An average of 2400 dorsal root fibres are myelinated. They are distributed in three populations: a population of small and poorly myelinated fibres with a mean diameter of 12±4μm (41%), and a third population of large calibre fibres with a mean diameter of 20±4μm (19%). These data will serve as an endpoint to a developmental study of myelogenesis of the spinal cord in the Monodelphis pristinus. (Supported by NSERC.)

469.11

ENCODING OF LIMB GEOMETRY BY SPINOCEREBELLAR NEURONS. G. Bosco and R.E. Poppele, *Dept. of Physiology, University of Minnesota, Minneapolis, MN 55455.

Only in the recent past has attention been devoted to the interactive features of second order sensory fibers. The DSCNT (Directional Selective Cells for Non-occlusion) represents a good model to investigate this process in a sensory-motor system because recent evidence suggests that it may convey to the cerebellum a complex signal related to parameters of movement for the whole limb, in particular the angular position of limb during. An average of 890 ventral root fibres are myelinated. They can be divided in two populations: small fibres having a mean diameter of 8±4μm (42%), and large fibres having a mean diameter of 18±4μm (58%). myelin sheaths included. An average of 2400 dorsal root fibres are myelinated. They are distributed in three populations: a population of small and poorly myelinated fibres with a mean diameter of 12±4μm (41%), and a third population of large calibre fibres with a mean diameter of 20±4μm (19%). These data will serve as an endpoint to a developmental study of myelogenesis of the spinal cord in the Monodelphis pristinus. (Supported by NSERC.)

469.12

BRAINSTEM DISTRIBUTION OF C-FOS IMMUNOREACTIVITY FOLLOWING REPETITIVE COUGHING IN DECEREBRATED CATS. C. Gertner, F. Portillo, M. Youkilis, J.J. Pariaitwice, L. White, M. URCI, CNRS 1832, St. Jerome, 13397 Marselle 20; (1) CNRS-LNB, 14302 Marseille Cedex 20, France (E-mail: KER)

Using the expression of the immediate-early gene fos as a marker of neuronal activation, we compared in the brainstem of cats the pattern of distribution of cells exhibiting a For-immunoreactivity (FLI) in response to repetitive coughing (50 coughs) in intact (E. A.) and stimulated animals (to that observed in shamed animals. In decerebrated, paralyzed and ventilated cats, 100 to 250 coughing episodes were elicited by stimulation (1-3 V, 4 Hz, 45 min) of superior laryngeal nerves (SLN), applied 4 hours after surgery was completed. Coughing was monitored on the phrenic and ilio-hypogastric nerves. Brainstem tissues were immunoprocessed using an antibody recognizing both the fos and fos-related nuclear antigens, and the avidin-biotin peroxidase complex. In the brainstem of stimulated cats, intense FLI was observed in various subnuclei (e.g. commissural, lateral, ventrolateral, medial and dorsal-medial) of the nucleus tractus solitarii, the area and hypoglossal nuclei, the nuclei of the accessory seventh cranial nerve, the inferior central (raphe) nucleus and the locus coeruleus. In these regions only very weak or FLI was observed. FLI in the medullary reticular formation and in the pontine lateral and medial parabrachial nucleus was only augmented in experimental cats.
649.13

RETROSPINAL NEURONS AND THEIR SPINAL PROJECTIONS IN LAMPRETS: A STUDY USING RETROGRADE TRACERS. N. Bissenpietra* and R. Dulac. Dep. de kinastrophopathie, USJAM HSC 3P4 and Centre de recherche en sciences neurologiques, Universite de Montreal, P.O. Box 6128, Station Centre Ville, Montreal, Quebec, H3C 3J7 Canada.

As in other vertebrates, lampry reticospinal (RS) neurons play an important role in the spinal somatosensory system. In the present study, reticospinal tracers were used to study the organization of RS lampry neurons within the different reticular nuclei and to characterize the spinal projections of these rhombencephalic neurons. Cobalt-lysine was applied as wide as the rostral end of the spinal cord (n=30), or applied isopotenolophically to label descending cells projecting to specific spinal cord rots (n=10). Another tracer, horseradish peroxidase (HRP), was applied in the 12th, 20th, 37th and 38th spinal segments and transported in vivo in order to determine the length of the axonal projections of RS cells (n=20). Reticospinal neurons were counted from serial sections on the best specimen for each injection site and their soma were measured. 3-D computer reconstructions were made of each tract made to a total of 35 RS neurons were counted on one side.

The posterior rhombencephallic reticular nuclei (PRRN) contains the largest number of RS cells (845), 21% (777) of which project to the contralateral spinal cord and are located mainly in the caudal rhombencephallic reticular nuclei (MRN) contains fewer RS neurons (339), 18% (60) of which project contralaterally and is found predominantly in the lateral basal plate. Of the 90 RS neurons counted in the contralateral rhombencephallic reticular nuclei (ARRN), 27% (24) project to the contralateral spinal cord. They are intermingled with cells projecting to the ipsilateral side. Finally, the mesencephalic reticular nucleus (MRN) contains 102 neurons, projecting their axons exclusively to the ipsilateral spinal cord. Isopotenolophic application of cobalt-lysine to the lateral tract on one side labelled, on both sides, a higher proportion of cells in the PRRN (635) than in the other nuclei (MRN=94, ARRN=1, MRN=1). Spinal cord injections at the 12th segment labelled 716 RS cells, with a steep (63%) decrease in the number of small (less than 20) and RS cells counted as compared to injections in the 2nd spinal segment. Injections at the 9th (n=1) and 8th (n=1) segments (tail) labelled a gradually decreasing number of neurons with only 19% (138) of all RS cells projecting to the level of the tail and distributed predominantly in the PRRN (96) and the MRN (33). Results from this study reveal a higher number of RS cells than previously reported, with a noticeable contralateral projection cells (Supported by MRC, FCAR, FRSQ and NCE for neural regeneration and recovery).

649.14

THREE-DIMENSIONAL DENDRITIC ANALYSIS OF RAT PHRENIC MOTORNEURONS DURING POSTNATAL GROWTH. K.G. Smithphoria, Y.S. Prakash and G.C. Stock. Departments of Anesthesiology and Physiology, Mayo Clinic, Rochester, Minnesota.

The rapid growth of diaphragm muscle fibers between postnatal day 21 (D21) and adulthood may also be reflected by growth of phrenic motorneurons and their dendritic arborization. Concurrent growth of the spinal cord may also occur and result in the ability of supraspinal motor output in order to properly integrate descending synaptic input. In the present study, qualitative and quantitative features of rat phrenic motorneuronal dendritic morphology were examined in D21 and adult rats. Cholera toxin B-fragment was used to retrogradely label phrenic motorneurons. Computer-assisted three-dimensional neuronomorphometry was used to statistically summarize dendritic branching patterns. While this study is preliminary, previous qualitative observations on rat phrenic motorneurons, several new features were highlighted. For example, contralateral projections of dendrites were more prominent in D21 animals than in adults. Dendritic diameter was smaller in D21 compared to adults. Total dendritic lengths were shorter at D21, and showed less variability compared to adults. Branching angles of D21 dendrites were also greater compared to adults, indicating a differential growth of dendrites during growth. This was also reflected by smaller receptive areas of motorneurons at D21. Receptive areas at D21 also showed greater variance compared to adults, indicating a differential growth of dendrites during growth.

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650.1
SUPERSPIRAL CONTROL OF CERVICAL SPINAL CORD MOTORNEURAL DENDRITE BUNDLES IN THE RAT. 
W.J. Anderson* and G. Bennett. Neurobiology Lab, Indiana Univ. Medical center, Terre Haute. IN 47803

Our laboratory has previously described motorneuronal columns in the cervical spinal cord of the rat into discrete dendrite bundles with continuity with the brain stem and thamic cord. This organization identified a midline, medial (which included the phrenic nucleus) and a lateral column (internal and external). These dendrite bundles in the rat have electronmicroscopic connections that are adenodendritic, endomesodromic, and somatotomeric. There are interconnections between these columns which are referred to as microbundles. This study will present data that this organization of dendritic columns function as a substrate for determining supraspinal axons which include serotonin, noradrenaerpine and thyroid hormone. Utilizing double immunocytochemical techniques, utilizing choline acetyltransferase and tyrosine hydroxylase, cholinergic and adrenaline releasing factor, and serotonin with different substrates from Chemicon Corporation, we have demonstrated that specific supraspinal axons specifically innervate motorneural columns with pericellular contacts, distributed along dendrite bundles in the longitudinal plane in a specific manner, and finally interact with microbundles which interact with the different columns. The distribution of each transmitter varies in its distribution to the various bundles. We hypothesize that this supraspinal control has specific control over different columns, and yet can influence all to varying degrees through their microbundle distribution.

650.2

Both cholinergic and glutamatergic neurons have been identified in the lateral tegmental bundle (LDT) and pedunculopontine (PPN) nuclei. Both cholinergic and glutamatergic neurons in LDT and PPN project to the pontine inhibitory area, an area that is involved in the control of REM sleep and slow frequency. The present study was designed to clarify if neurons in LDT and PPN co-contain ACh and glutamate, utilizing choline acetyltransferase (ChAT) and glutamate immunohistochemical techniques. Three cats were perfused with saline followed with 3% paraformaldehyde and 0.25% glutaraldehyde in phosphate buffer solution, pH 7.4. The tissue was cut at 50 μ and alternate sections were processed with the following 1) ChAT 2) glutamate and 3) ChAT and glutamate. We found that neurons in both LDT and PPN co-contain glutamate and acetylcholine. However, the percentage of cholinergic neurons co-containing both transmitters was higher in PPN than in LDT. 78% of cholinergic neurons in PPN were double labeled with glutamate, while only 44% of neurons in LDT contained both transmitters. We suggested that the neurons in LDT and PPN that co-contain Ach and glutamate may play an important role in REM sleep control.

650.3
COLOCALIZATION OF SUBSTANCE P IN SEROTONERGIC AFFERENTS TO THE HYPOGLOSAL NUCLEUS. S, Makser* and J.N. Henry. Center for Sleep and Respiratory Neurobiology and Pulmonary and Critical Care Division, Department of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

The serotonergic (5HT) innervation of the hypoglossal nucleus (M12) originates from the caudal raphe nuclei (CRN). Non-SHT neurons in these nuclei also project to the M12. Substance P (SP) cells are co-localized with 5HT. We sought to determine the presence of SP in non-5HT afferents to the M12. Rhodamine microphores (Rt; 100ml) were injected into the M215 of anesthetized rats, who survived 6-9 days before sacrifice. Rats were reanesthetized and injected intraventricularly with 100 μg of 5HT. Following perfusion and fixation, sections (32 μm) were cut through the entire brainstem. In cases with the Rh injection site restricted to the M12, every seventh or tenth section was processed for immunofluorescence for 5HT (rabbit anti-5HT, 1:2000; AMCA-conjugated goat-anti-rabbit, 1:500) and SP (rat monoclonal anti-SP, 1:200; FITC-conjugated goat-anti-rabbit, 1:100, Retagrade labeling by RH was observed to label 3% of M215 afferents, while SHT or SP immunofluorescence was present in all major groupings of SHT or SP somata, respectively. Within the median tegmental field, most SPT neurons also contained SHT. Of the neuronal afferents projecting to the M12, most (>79%) of the SHT-immunoreactive neurons also contained SP, and these triple labeled cells were present mainly within the CRN. Very few (<10%) of the non-SHT afferents to the M12 were SP positive. These observations suggest that the SP projections to the M12 are a subset of the SHT projections to the M12, and that SP neurons account for a very small proportion of the non-SHT afferents to the M12. (Supported by SCOR HL-42350).

650.4
A STUDY OF THE TRANSMITTER SYSTEMS MEDIATING TECTOSTRIATAL INPUTS IN LAMPERYS. I. Zec, and R. Dubois. Dep. de kinesiologie, Université du Québec à Montréal, H3C 1P8 and CRIN, Université du Montréal, H3C 3J7, Canada.

It has been shown by Uhl et al. (Behav. Brain Res. 50:107-110, 1993) that illumination of a lateral eye in lampreys induced a turning movement away from the light source. Anatomical studies have revealed that the retinae of lamprey contain two distinct classes of photoreceptors: one sensitive to blue (Cavallini & Rubenson, J. Comp. Neurol. 171:465-480, 1977). However, little is known on the interactions between visual inputs and motor systems in lampreys. In a previous study, we identified populations of neurons in the caudal raphe nuclei (CRN) that project to the midbrain tegmental region (M12) and the pontine reticular region (PRRN) in lampreys. These neurons contain immunoreactive 5′-OH indoleamines which are known to play a key role in descending modulatory control. We have also shown that microinjection of the cerebellum, in the in vitro isolated brainstem-spinal cord preparation, evoked mixed excitatory and inhibitory responses in neurons. This study was aimed at characterizing these tectostriatal inputs further. Large Mller cells of the M12 received strong excitatory inputs, while some RS neurons in the caudal PRRN received predominantly inhibitory inputs. To identify the transmitter systems involved, CNOX (10 μM) was added to the perfusion, and both the excitatory and inhibitory inputs were abolished, suggesting that AHPa receptors were involved in mediating these responses and that inhibition is due or oligogenic. Strauschny (5M) completely abolished the inhibitory responses, indicating that glycine is the inhibitory transmitter. Perfusion with the preparation with Mg2+-free Ringer's, increased the duration of the excitation and induced an inhibition by 100 μM A5P or when normal Mg2+ concentrations were restored. NMDA receptors are thus present in this pathway. In conclusion, we have shown that excitatory and inhibitory inputs from tectum to midbrain nuclei are similar to those reported in rats (Furman et al., J. Exp. Brain Res. 21:19-44, 1974). Moreover, this study indicates that within tectosubicular pathways of lampreys, excitation is mediated by excitatory amino acids and inhibition by glycine. Funded by NRC Canada, FCAR and FRSQ Quebec.
SYNAPTIC INTERACTIONS OF SUBSTANCE P IMMUNOREACTIVE (SP) NEURON TERMINALS WITH HYPOGLOSAL (XII) MOTEURONS WHICH INNERVATE THE INTRINSIC MUSCLES OF THE TONGUE: AN ELECTRON MICROSCOPIC DUAL LABELING STUDY* P.J. Gaál1, T.A. Johnstone2, M. Ghiradelli3, and V.J. Massi4. Dept. of Pharmacology and Veterinary Services. Harvard University. Med. School, Boston, MA. We have found tetramethylbenzidine chromogen. The intrinsic muscles of the tongue play important roles in speech and respiration. Little is known of the central mechanisms regulating XII motoneurons. To elucidate which afferent neurons and neurotransmitters modulate functionally associated subgroups of XII motoneurons, we have employed an electron microscopic dual labeling technique. Cholera toxin-biosorbed peroxidase and antiserum to substance P have been incubated into the intrinsic tongue muscles of the cat. The animals were sacrificed 48 hr later and sections were processed histochemically for the visualization of HRPs using tetramethylbenzidine as the chromogen. retrogradely labeled neurons were found predominantly ipsilaterally in the intermediate and ventromedial subdivisions of XII particularly at the level of the rostral pole of the area postrema. Sections were processed for the simultaneous immunocytochemical localization of SP using dianaminoazide as the chromogen. SP immunoreactivity was found in nerve terminal processes associated with large dense core vesicles. Some of these terminals made synaptic contact with proximal dendrites and perikarya of labeled XII motoneurons. These data indicate that SP affects modulate the activity of XII motoneurons which control the function of intrinsic tongue muscles. Supported by NIH HL44922, & Howard Univ. Grad. Sch. Collab. Core Prog.


Serotonergic systems exert important modulatory effects on sensory neurons and motoneurons in the mammalian spinal cord. These effects may be inhibitory or facilitatory, are mediated by synapses and receptors distributed over the surface of the target neurons. We were interested to determine if an important sensory feedback pathway providing proprioceptive information to the cerebellum, the dorsal spinocerebellar tract (DSC), received direct serotonergic input and to what extent such input was distributed over the proximal versus distal dendrites of DSC cells. Identified DSC cells were intracellularly stained with horseradish peroxidase (HRP), and 5-HT immunoreactive varicosities in contact with the cells were revealed by immunohistochemistry using an antibody raised in guinea pigs against keyhole limpet hemocyanin-conjugated 5-HT. At the light microscopic level, 5-HT contacts were observed on the soma and all regions of the extensive dendrites of stained DSC cells. However, the density of 5-HT contacts was much lower than previously observed on spinal motoneurons, suggesting that the DSC is not subject to particularly powerful descending serotonergic influences, thereby allowing the system to faithfully and rapidly relay its information regardless of activity in other systems. Supported by NIH grant NS25547.


Glycine is a major inhibitory neurotransmitter in the ventral horn of the spinal cord, exerting powerful effects over neural elements involved in motor behavior. Here we evaluated quantitatively various synaptic parameters related to glycinerergic terminals on the cell somas of α-motoneurons (α-MNs) and Renshaw cells (RCs). We assessed glycine receptor presence using electron microscopy pre-embedding immunocytochemistry with antibodies directed against gephrin, a protein associated with postsynaptic glycine receptor clusters. α-MNs were identified by their size, location and characteristic synaptology. RCs were identified by their location in ventral lamina VII and their distinctive gephrin-immunoreactivity (geph-ir). Overall synaptic covering was similar in α-MNs and RCs (45-45% of the available somatic membrane), with geph-ir terminals being the most abundant type on both RCs and α-MNs. While, terminals with geph-ir synapses constitute about 90% of the synaptic contacts in RCs, they constitute only 40-45% in α-MNs. An average of 87% of all terminals that contacted RC somas displayed geph-ir while this value was 49% for α-MNs. All geph-ir terminals were of the F-type, and had similar ultrastructural features on RCs and α-MNs. The percentage of apposition area occupied by postsynaptic geph-ir was larger in RC somas: 50-40% compared to 50-20% on α-MNs. The extension of postsynaptic geph-ir was almost identical to the extent of the opposed presynaptic active zone. In presynaptic active zones larger and the postsynaptic receptor regions are usually more intensely immunoreactive than those on α-MNs somas. Supported by NIH grant NS25547.


Spinalized cats exhibit variable degrees of weight bearing and locomotor ability in their hindlimbs, and these parameters can be differentially affected by training and certain pharmacological manipulations. To determine if the variability between animals is correlated with different patterns of neurochemical reorganization we studied the distribution of immunocytochemical markers for a variety of neurotransmitters and receptors involved in descending and segmental control of motor function, in cats that had undergone different training paradigms and exhibited different types of motor behavior following transection at T12-T13. Unlike controls, immunoreactivity for serotonin, tyrosine hydroxylase, and thyrotropin releasing hormone was completely absent in all cats in segments below the lesion site, indicating that the transections were complete in all cases. Interestingly, CGRP immunoreactivity was decreased in the ventral horn, suggesting some direct or indirect supraspinal influence. A similar result was observed for substance P labeling. There was no obvious rearrangement or expansion of markers for likely segmental transmitters (e.g. GAD, glycine receptors) to occupy sites vacated by the descending systems. It appears that the difference in locomotor capability among cats is the result of more subtle changes in circuitry or transmitter/receptor expression in spinal neurons. Supported in part by NIH grants NS16333 and NS25547.
561.1 
SERTONIN (5-HT) REDUCES AN AFTERHYPERPOLARIZATION IN NEONATAL RAT MOTONEURONS THROUGH INHIBITION OF N- AND P-TYPE CALCIUM CHANNELS. D.A. Batlle*, M. Umeno* and A.J. Berger, Dept. of Physiology and Biophysics, University of Washington, Seattle, WA 98195. We have shown that 5-HT reduces input-output gain of neonatal rat hypoglossal motoneurons (HMs) via a decrease in synaptic back-pulse generation of Ca⁺-dependent afterhyperpolarization (AHP) (Neuroreport 14: 163, 1994). To determine if the decreased AHP is mediated by decreased Ca⁺ influx, we tested the effect of 5-HT on Ca⁺ currents in neonatal motoneurons. The presence of Ca⁺-selective micro-electrode preparation of Ca⁺-dependent AHP in hypoglossal motoneurons (HMs) was confirmed, as a decrease in synaptic back-pulse generation of Ca⁺-dependent AHP (8-10% AHP) was blocked by Co⁺ in the recording bathing solution. Application of 5-HT (10⁻⁵M) decreased synaptic back-pulse generation of Ca⁺-dependent AHP (20% AHP) and did not affect the rectification of Ca⁺ currents (20% rectification at 100mV). L-type Ca⁺ currents were completely blocked by La⁺ (2mM) and N-type Ca⁺ currents were partially blocked by a 10⁻⁵M solution of 2-chloroadenosine (2-CA), a blocker of N-type Ca⁺ channels. These results indicate that 5-HT decreases synaptic back-pulse generation of Ca⁺-dependent AHP in neonatal HMs and, in this way, 5-HT enhances the excitability of HMs. (Supported by NS14857 and Francis Families Foundation). 

561.2

AT LEAST TWO DISTINCT IONIC MECHANISMS UNDERLIE THE RESPONSE OF HYPOGLOSSAL MOTONEURONS (HMs) TO NOREPINEPHRINE. M.A. Parker*, D. Belin**, and A.J. Berger. Depart. of Physiology and Biophysics, University of Washington, Seattle, WA 98195. The in vitro response of HMs to norepinephrine (NE) mimics their response to thyrotropic releasing hormone (TRH), i.e. depolarization with an increased input resistance (Ra) recorded in current-clamp, or development of an inward current with decreased input conductance in voltage-clamp (J. Neurophy. 68:1733, 1992, Soc. Neurophy. Abstr. 18:512, 1992). The NE current, like the TRH current, reversed near-100 mV. In addition, the effects of NE on HMs are blocked by TRH in the perfusing artificial cerebrospinal fluid (ACSF, Soc. Neurophy. Abstr. 19:988, 1993), suggesting that these two neuromodulators are not acting via similar mechanisms of action in HMs. Since the TRH-mediated increase in Ra is elicited by substitution of barium (Ba²⁺) for calcium in the ACSF, while the norepinephrine is not, we tested whether the same hold true for the response of HMs to NE. We found that the increase in HMs Ra elicited by NE was reduced from 50% in control to only 3% in Ba²⁺-ACSF, despite the cells' continued ability to exhibit a depolarizing response (n=7). After return to Ba²⁺-free ACSF, HMs again showed increased Ra in response to NE. To investigate the ionic mechanism responsible for the Ba²⁺-resistant depolarizing response, we substituted choline chloride for sodium chloride in the ACSF. Within ten minutes of switching into a Ba²⁺- and choline-substituted ACSF, HMs no longer depolarized in response to NE (n=4). After longer periods of time the response became a hyperpolarization. In choline-substituted ACSF without Ba²⁺, a depolarization with increased Ra could still be elicited by NE; but recovery from the depolarization was prolonged, the potential taking longer to return to baseline (n=3). We conclude that NE depolarizes HMs by at least two ionic mechanisms: reduction of a Ba²⁺ sensitive inward current (n=1), followed by a Ba²⁺-resistant depolarizing current carried predominantly by sodium ions. (Supported by HL-46507).

561.3

ADENOSINE MODULATES EXCITATORY SYNAPTIC TRANSMISSION TO RAT HYPOGLOSSAL MOTONEURONS. M.C. Bellingham* and A.J. Berger. Dept. Physical & Biophysics. Univ. Washington, Seattle, WA 98195. Adenosine-mediated modulation of excitatory inputs may be especially significant in hypoxic responses of HMs, as extracellular CNS adenosine levels rise many-fold under these circumstances. To investigate this modulation, intracellular recordings were made from adult rat hypoglossal motoneurons (HMs; n=25) in brainstem slices. Electrical stimulation lateral to the hypoglossal motor nucleus evoked short-latency (0.6±0.1 ms) excitatory postsynaptic potentials (EPSPs). These EPSPs were markedly depressed or abolished by bath application of kynurenic acid (1 mM; n=7), showing that they were glutamatergic. Bath application of adenosine receptor agonists 2-chloroadenosine (2-CA, 1 µM) and 2-chloroadenosine (2-CA, 1 µM) significantly reduced EPSP amplitude to 42 ± 5% (n=6) and 76 ± 7% (n=6) of control, respectively. The adenosine receptor agonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 0.1-1 µM) significantly increased the EPSP amplitude to 124 ± 14% of control (n=9), and blocked EPSP reduction by bath or local application of 2-CA or 2-CA (n=7). 2-CA, 2-CA and DPCPX did not significantly alter the AMPA amplitude of the mEPSCs. These data indicate that adenosine-mediated modulation of excitatory inputs to rat HMs are mediated by adenosine A1 receptors, most probably at a presynaptic site. Modulation of presynaptic N-type calcium channels does not appear to be essential for the adenosine-mediated effects, as presynaptic A1 receptors reduce excitatory synaptic transmission to HMs. (Supported by NS14857, HL-4657).

561.4

PRESYNAPTIC INHIBITION BY SEROTONIN OF GLYCINEnergIC INHIBITORY POSTSYNAPTIC CURRENTS (IPSCs) IN RAT MOTONEURONS. M.U. Umeno* and A.J. Berger. Dept. of Physiology and Biophysics, University of Washington, Seattle, WA 98195. Serotonin (5-HT) plays an important role in the neuronal function of synaptic modulation. Using a thin-slice preparation, we found that 5-HT presynaptically inhibited unitary glycine IPSCs of hypoglossal motoneurons (HMs). After intracellular injection of 5-HT (10⁻⁵M) into the terminal of an extracellularly stimulated synapse and recording of motoneurons in rat brainstem. One possible mechanism for presynaptic inhibition is K⁺ channel activation and/or voltage-activated Ca⁺ channel inhibition at the presynaptic terminal. In this regard, the 5-HT₁B and/or 5-HT₃ receptors, inwardly rectifying K⁺ channels and voltage-activated Ca⁺ channel were blocked by Co⁺ and Ni⁺. These results indicate that 5-HT presynaptic inhibition may be a K⁺ channel and voltage-activated Ca⁺ channel blocking mechanism. (Supported by NS14857).
651.7 DESCENDING SEROTONERGIC CONTROL OF CROSSED GROUP II INHIBITION IN THE SPINAL CORD OF THE CAT. N.C. Aggelopoulou1, B.W. Clarke2 and S.A. Edgeley1. 1Department of Anatomy, University of Cambridge and 2Department of Clinical Neurosciences, University of Nottingham, United Kingdom. SPONS: Brain Research Association.

Stimulation of hindlimb group II muscle afferents inhibits many contralateral hindlimb motoneurons. This crossed inhibitory reflex depends on a descending system as it disappears on section of the spinal cord. We have tested the hypothesis that crossed inhibition is dependent on serotonin transmission.

Experiments were carried out in four cats under chloralose general anaesthesia. Intracellular recordings were obtained from hindlimb motoneurons. Electrical stimulation of the contralateral quadriceps nerve at a strength sufficient to activate group II afferents (5T) elicited IPSPs in the majority (44/46: 96%) of gastrocnemius/soleus and hamstring motoneurons. The incidence of IPSPs was reduced after spinal transection at the thoracic level (94/100: 22%) and the IPSPs were smaller. After spinalization, intravascular administration of (+)-5,7,8-trifluoro-5H-tryptamine (8-OH-DPAT, Research Biochemicals), a selective agonist of 5HT1A receptors (dose 0.1-1.2 mg/kg, i.v.) restored the crossed inhibition of many motoneurons (44/46:85%) within 5-15 min. The effect of 8-OH-DPAT was antagonized with a latency of 10-20 min by the selective 5HT2 antagonist (+)-WAY-100135 (a gift of Wyeth Research UK) injected intravenously at a dose of 3.7-3.8 mg/kg, which markedly reduced the incidence of IPSPs in motoneurons (104/45: 22%).

The simplest explanation of these results is that a descending pathway acting via 5HT1A receptors permits crossed group II inhibition to operate. Supported by the Wellcome Trust and MRC (UK).


NADPH-diaphorase staining is thought to reveal neurons which use nitric oxide as a neuromodulator (J. Comp. Neurol. 324:410, 1993). Cholinergic neurons of the pedunculopontine tegmental nucleus (PPT) projecting to the mPRF are NADPH-positive, and electrical stimulation of PPT neurons increases mPRF ACh release (Am. J. Physiol. 264:R544, 1993). Therefore the present study is testing the hypothesis that inhibition of nitric oxide synthase with Nω-nitro-L-arginine (NLA) will result in state-dependent increase of mPRF ACh release. A cat was chronically implanted with electrodes for monitoring sleep and wakefulness. For each experiment, a microdialysis probe was stereotaxically placed in the mPRF for simultaneous recovery of ACh and delivery of either Ringer solution or 10 mM NLA. Ten minute dialysate samples (n=2) were collected during waking (n=4), non-REM sleep (n=4), and REM sleep (n=6). Samples were analyzed for peak of ACh using HPLC. ACh levels (mean±SD) during waking with NLA dialysis (0±10.11) were significantly less (n=23, df=44, p=0.002) than ACh levels with Ringer alone (0.34±0.29). Likewise, during REM sleep ACh levels with NLA dialysis (0.12±0.11) were less (n=29, df=46, p=0.002) than ACh levels with Ringers (0.20±0.05). During REM sleep, however, there was no significant difference (p=0.777) in ACh levels between NLA dialysis (0.34±0.29) and dialysis with Ringers (0.38±0.12). These data suggest nitric oxide may modulate state-dependent cholinergic neurotransmission within the mPRF. Support: Department of Anesthesia, HL-40851 (R31).

651.9 SEROTONIN AND NOREPINEPHRINE MODULATE EXCITATORY AMINO ACID RECEPTOR CURRENTS IN ACUTELY ISOLATED RAT VENTRAL HORN NEURONS. S.C. MacDonald, S. Hochman, and I.M. Jordan. Dept. of Physiology, University of Manitoba, Winnipeg, MB, R3E0W3.

It has previously been reported that serotonin (5-HT), and norepinephrine (NE) and N-methyl-D-aspartate (NMDA) are involved in locomotor-like activity in the neonatal rat preparation. We hypothesize that neurotransmission of excitatory synaptic transmition by monoamines plays a role in the generation and control of locomotion. Our goal is to examine the mechanisms of this modulation at a single cell level. We examined the effects of two monoamines, 5-HT and NE, on excitatory amino acid (EAA) currents in the ventral horn. Whole-cell voltage clamp recordings were obtained from acutely dissociated neurones from the area of the spinal cord where locomotor-related neurones are found. Local perfusion of NMDA and kainate evoked inward currents. These currents increased or decreased in the presence of either 5-HT or NE. Within single neurons, NE and 5-HT could be added concurrently to test EAA receptor subtype, whether facilitatory or inhibitory. Kainate currents were examined in 38 cells and were depressed in 26% of cells and potentiated in 45%. There was a mean current increase of 77.9±54.9% and 66.9±40.3% and decrease of 37.9±17% and 21.9±3.5% in the presence of 5-HT and NE respectively. Similarly, out of 28 cells examined with NMDA, 18% of cells were potentiated and 54% were depressed. Mean increases in current were 27.9±22.7% and 33.8±17.2% and decreases 16.6±11.9 and 16.9±11.4% for 5-HT and NE respectively. Preliminary results indicate that 5-HT1A and 5-HT2A nonadrenergic receptors contribute to the observed effects. These results suggest that 5-HT and NE can modulate excitatory synaptic transmission at the level of the postsynaptic receptor. Supported by MRC. S.C.M. is a Network of Centres of Excellence Trainee. (1Cazanavet et al, Neuroscience, Lett., 1990)

651.11 FAILURE OF INTRATHecal NIMODIPINE TO INCREASE SPINAL CORD BLOOD FLOW. H. Inamura and C.H. Talbot*. Division of Neurosurgery and Playfair Neuroscience Unit, Toronto Western Division, The Toronto Hospital, University of Toronto, Toronto, Ontario M5G 2G8, Canada.

We have demonstrated that intravenously administered calcium channel antagonist nimodipine produced an increase of spinal cord blood flow (SCBF) in normal and injured rats. However, in the injured rats, due to the hypotension caused by both cord trauma and the vasodilator effects of nimodipine it was necessary to counteract the hypotension and maintain the mean arterial blood pressure. Nimodipine has high lipid solubility and penetrates well into the central nervous system, so the present study was performed to investigate whether intrathecally infused nimodipine could increase the SCBF in normal rats. Male Wister rats anesthetized by alpha-chloralose and urethane had a laminectomy from C1 to T1 and a silastic tube was inserted into the subarachnoid space via the atlantooccipital membrane to the C6 level. We administered intrathecal nimodipine at a concentration of 0.05 mg/kg, n=5; 0.2 mg/kg, n=5 and placebo (n=5), and measured SCBF at C7T1 with the hydrogen clearance method before infusion, during infusion and 30 minutes after infusion of the drug. Neither 0.05 nor 0.2 mg/kg of nimodipine increased SCBF during infusion (f=12.13; p<0.05) or after infusion (f=12.13; F=13.1; p<0.001). Although 0.2 mg/kg nimodipine caused a 26.4% decrease in blood pressure at the end of infusion (p<0.006), it recovered to 85.2% of preinfusion value at the end of the experiment (p>0.1). These data suggest that intrathecal nimodipine at these doses cannot increase spinal cord blood flow.
652.1


We have previously shown that dopamine is present in cell bodies and processes in the lamprey spinal cord and that dopamine modulates fictive swimming (Neurosci. Lett., 163:23-26,1994). To further investigate the actions of dopamine, we examined the effects of dopamine at several levels: (i) in fictive swimming lamprey, 2) in network, cellular, and synaptic properties in the isolated spinal cord, and 3) on ionic currents in isolated spinal neurons. The experiments were done on adult sea lampreys (Petromyzon marinus) and adult silver lampreys (Ichthyomyzon asiaticus).

Injection of the dopamine receptor agonist, SKF 38393, decreased the cycle period of swimming, an effect similar to that of low concentrations (<1µM) of dopamine during fictive swimming. The D1 dopamine receptor agonist administered alone had no effect on fictive swimming. Injection of the dopamine receptor antagonist, 2-iodo-3-PPP, had no effect. Dopamine reduced the late after-spike hyperpolarization in motoneurons (MN), primary sensory dorsal cells (DC), stretch receptors, and afferent dorsal roots during fictive swimming, while the D2 dopamine receptor agonist had no effect. We conclude that dopamine acts through the D1 dopamine receptor to modulate fictive swimming. In our experiments, the dopamine receptor agonist administered alone had no effect on fictive swimming. Injection of the dopamine receptor antagonist, 2-iodo-3-PPP, had no effect. Dopamine reduced the late after-spike hyperpolarization in motoneurons (MN), primary sensory dorsal cells (DC), stretch receptors, and afferent dorsal roots during fictive swimming.

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652.2


We used photo-ablation to test the involvement of commissural interneurons in fictive swimming in the isolated spinal cord. Spinal interneurons were retrogradely labeled by applying a mixture of fluorescein- and eosin-dextran amine to a transverse hemisection of the midbody spinal cord of adult sea lampreys. After 1 to 5 weeks of survival, fictive swimming was induced in 8-segment lengths of spinal cord with N-methyl-DL-aspartate. An argon laser beam with a diameter of one-half the width of the spinal cord was moved along the spinal cord caudal to the dye application site, allowing the laser beam to focus adjacent to the spinal cord. Retrogradely labeled interneurons decreased the cycle period of swimming, an effect similar to that of low concentrations (<1µM) of dopamine during fictive swimming. The D1 dopamine receptor agonist administered alone had no effect on fictive swimming. Injection of the dopamine receptor antagonist, 2-iodo-3-PPP, had no effect. Dopamine reduced the late after-spike hyperpolarization in motoneurons (MN), primary sensory dorsal cells (DC), stretch receptors, and afferent dorsal roots during fictive swimming, while the D2 dopamine receptor agonist had no effect. We conclude that dopamine acts through the D1 dopamine receptor to modulate fictive swimming. In our experiments, the dopamine receptor agonist administered alone had no effect on fictive swimming. Injection of the dopamine receptor antagonist, 2-iodo-3-PPP, had no effect. Dopamine reduced the late after-spike hyperpolarization in motoneurons (MN), primary sensory dorsal cells (DC), stretch receptors, and afferent dorsal roots during fictive swimming.

652.3

INTERSEGMENTAL COORDINATION AFTER CHRONIC AND ACUTE LESIONS IN LAMPRY SPINAL CORD ASSESSED DURING FICTIVE SWIMMING. L. Grun*, T. Kiemel, D. Liao and A.H. Cohen*. Department of Zoology, University of Maryland, College Park, MD 20742.

Previously, we reported the use of correlation analysis in the isolated lamprey spinal cord to demonstrate that during fictive swimming there is strong intersegmental coupling. We define strong coupling in terms of the speed with which the bursting returns to its regular frequency after external perturbation. In this analysis we extract the cross-correlation of post-spike intervals between the delay pair between segments. High cross-correlation of periods and low auto-correlation of delays indicated strong coupling. We compared the results to simple models of coupled non-linear oscillators to illustrate the plausibility of the conclusion. Here we extend the method to spinal cords previously either acutely or chronically lesioned. We performed experiments that were either to the lateral or the medial fiber tracts of the entire spinal cord. At the time of testing, in partially lesioned animals acute lesions of the formerly spared fibers were often added to the chronic lesions to examine the function of regenerated fibers in the absence of the lesioned fibers. Chronically lesioned cords were compared to acutely lesioned healthy cords. The analysis of the acutely lesioned spinal cords revealed moderate to strong coupling of strength either medial or lateral tract coordinating fibers. However, there was evidence that the regular fibers were capable of strong or moderate strength coupling even after 10 months of recovery. Only in the presence of spared fibers was any strong coupling found. In only two cases was there even a hint of moderate strength coupling of regenerated fibers with this method of analysis. Thus, the regeneration, at best, seemed to restore only weak or perhaps occasionally moderate strength coupling among the segments.
A single action potential elicited in one of the two Mauthner axons (M-axon) while a fish is "fictively" swimming can dramatically reset the swimming rhythm. This is due to the ability of these neurons to identify cellular elements within the M-cell network that may subserve this resetting phenomenon. Firing both M-axons simultaneously results in cranial motor output to spinal motor output because the spinal commissural inhibitory interneurons in the M-cell network block firing of motoneurons and excitatory interneurons. We fired both M-axons simultaneously to determine if detection of firing in both M-axons was not sufficient to assess whether the resetting of the swimming rhythm could occur in the absence of spinal motor output from the escape network. Fictive swimming was elicited by stimulation of the midbrain of de cerebrate, paralyzed goldfish and the motor pattern was monitored by recording extracellularly from branches of ventral roots. A single intracellularly elicited action potential was initiated in each M-axon simultaneously (within 0.1 ms of each other) during bouts of fictive swimming. Simultaneous firing of both M-axons could reset the swimming rhythm indicated by a shift in the midpoints of swim- ming bursts post-axon stimulation relative to when they would be expected to occur based upon the pre-stimulation burst pattern. This result indicates that the resetting of the swimming rhythm can occur in the absence of spinal motor output from the Mauthner cells and suggests that the commissural inhibitory interneurons in the M-cell network contribute to the resetting. Support: NIH NS26539 (JRF).

652.9 CONFOCAL IMAGING OF RESPONSES IN POPULATIONS OF IDENTIFIED MOTONEURONS DURING ESCAPE BEHAVIORS OF INTACT ZEBRAFISH. J.R. Fetics* and D.M. Mcgillicuddy. Dept. of Neurobiology, Univ. of Minnesota, St. Paul, MN 55108.
The mechanisms that contribute to rostrocaudal phase lags in spinal locomotor networks in larval lamprey, A. hectori and A. rostrata, were studied with phaseolus vulgaris leucoagglutinin (PHA-L) retrograde tracing. PHA-L, a lectin with a 100% specific binding to neurons, was used to fill intracellularly many motoneurons in the ventral spinal cord (2-10 cells). PHA-L tracing was done under anesthesia with injected zolazepan maleate. Tracing was done under an upright fluorescence microscope (Olympus BX51-W, Lumar, 10x) equipped with a high resolution camera (Kodak Megaplus Color Camera). Figure acquisition was done with Scion Image. Spinal cord was dissected in Tris-buffered saline with 10% fetal bovine serum and held on a dissection ring. After dissection, the cord was placed in 40% paraformaldehyde. After 1 hour fixation, the cord was transferred to 30% sucrose in Tris buffer for 12-24 hours before being embedded in O.C.T. freezer medium (Tissue Tek). Transverse sections were cut with a cryostat to 12-15μm and mounted on slides. After mounting, sections were pre-incubated in blocking solution (0.3% Triton X-100, 1% normal goat serum, 0.2% FCS in 0.9% saline) for 1 hour at room temperature. The blocking solution was then replaced with blocking solution containing primary antibody (rabbit polyclonal, 1:500) in 0.3% Triton X-100, 1% normal goat serum, 0.2% FCS, and incubated for 24 hours at 4°C. After incubation with primary antibody, the sections were washed three times in PBS (3 minutes each) and incubated in secondary antibody (Alexa Fluor 488 or 594, goat anti rabbit, 1:2000 in 0.3% Triton X-100, 1% normal goat serum, 0.2% FCS, and PBS for 2 hours at room temperature). Sections were washed with PBS (3 times 3 minutes) and mounted on glass slides using Vectorshield (Vector Laboratories). Sections were observed with a Leica DM-RB microscope and images were captured with a Cooke Sensicam camera. Images were analyzed using Scion Image software.

652.10 MECHANISMS CONTRIBUTING TO ROSTOROCAUDAL PHASE LAGS IN SPINAL LOCOMOTOR NETWORKS IN LARVAL LAMPREY. A. Heegard* and J. Mcgillicuddy. Dept. of Neurobiology, Univ. of Minnesota, St. Paul, MN 55108.
The mechanisms that contribute to rostrocaudal phase lags in larval lamprey (Petromyzon marinus) were investigated in partitioned in vitro spinal cord preparations and by computer modeling. In in vitro preparations, swimming activity was elicited by chemical microstimulation in brainstem locomotor areas. Short distance coupling, Stycine applied to the rostral spinal cord (n=11) converted left-right alternation to synchronous swimming in the rostral 20% of the spinal cord (Neurosci. Abstr. 19:349, 1993). Stycine applied to the caudal spinal cord resulted in a reduction of continuous activity in the caudal 20% of the spinal cord with little effect on the left-right phase of the locomotor activity in the rostral cord. These results suggest that ipsilateral excitatory coupling is stronger in the ascending direction. Computer modeling confirmed the biological results and suggested that a dominant descending coupling contributes to rostrocaudal phase lags. Oscillatory frequency, amplitude, and long distance coupling. Cycle times of locomotor activity were examined in sections of the spinal cord when low-calcium Ringer's solution was applied to the rostrolateral section of the cord. Cycle times of the isolated caudal cord were similar to cycle times in the entire cord (n=7), but did appear to depend on the length of the rostral segments and the intrinsic cycle time. Cycle times of the isolated caudal cord were more closely related to the overall cycle times (6 of 7). Low-calcium Ringer's solution applied to the rostral spinal cord resulted in a reduction in the phase lag between the rostral and caudal cord compared to control. One interpretation of these results is that a frequency gradient along the cord contributes to rostrocaudal phase lags, and that long-distance coupling is symmetrical or perhaps stronger in the ascending direction which, by itself, would tend to reduce intersegmental phase lags. We are currently conducting experiments and performing computer modeling to investigate the combined effects of short distance coupling, long distance coupling, and oscillatory frequency gradient on rostrocaudal phase lags. (Supported by NIH NS29043, APA MB1 9108).

652.11 PEP TIDERGIC MODULATION OF THE SPINAL NETWORK FOR LOCOMOTION IN LAMPREY BY NEUROTENSIN AND SOMATOSTATIN. P.T. BARTHE and S. GRILLIER* Department of Neurosciences, the Nobel Institute for Neurophysiology, Karolinska Institute, S-17177 STOCKHOLM - SWEDEN.
Spinal neurons containing the neuropeptides somatostatin (SS) and neurotensin (NT) occur in lamprey. To gain insight into the ionic mechanisms and the functional role of these two peptides the effects of somatostatin and neurotensin were studied both in the cell culture and in the network level. Fictive locomotion was elicited by both application of the glutamate agonist NMDA (50-150μM) and by recordings from ventral roots. When both NT and SS (10-100 μM) reversibly slowed the NMDA-induced rhythmic activity by 2-5%. Furthermore, SS induced a reduction of the burst proportions (burst/duration/cycle period), whereas NT did not alter the burst pattern. In some cases the swimming activity became irregular with higher doses of NT. NT induced a depolarization (4.5-6.5 mV) of motoneurons and interneurons which were followed after blockage of voltage sensitive sodium channels with TTX and after removal of calcium. The fast and slow phases of the afterhyperpolarization were not affected. SS induced a hyperpolarization of spinal neurons and a decreased their firing frequency in response to depolarizing current pulses. SS also reduced the firing of interneurons and motoneurons recorded during fictive locomotion. It also induced a slowing of TTX-resistant, NMDA-induced membrane potential rhythmic oscillations. This study demonstrates that NT and SS modulate spinal activity by two different mechanisms, which both lead to a slowing of the swimming activity but with a different control of the burst parameters.

In the lamprey, a simple eel-like vertebrate, swimming is produced by bursts of activity alternating between the left and right sides, with a frequency from 0.25 to 10 Hz in combination with a constant phase lag between consecutive segments resulting in a wave travelling down the body pushing the animal forward through the water. The swimming rhythm generating network has previously been modelled as a chain of coupled oscillators, due to its ability to produce fictive swimming with only short sections of intra spinal cord. Here we introduce a continuous network model, using populations of neurons that synchronously spread rostrally and caudally along the spinal cord. Excitatory interneurons and caudally projecting crossed inhibitory interneurons are included in the network. The neurons are modelled according to a Hodgkin-Huxley type formalism. Excitatory synapses extend seven segments both rostrally and caudally with strengths decreasing linearly with distance. The same connection strategy is used for crossed inhibitory synapses with the addition that they extend 20 segments caudally. A network consisting of 2600 neurons and over 700,000 synapses has been used as a basis for the simulation. This network model produces stable forward swimming over a wide range of frequencies and can exhibit backward swimming by increasing excitability to the caudal segments. In order to further analyze the detailed contribution of different elements in the network we have studied different reduced networks. This has provided a deeper understanding of the different mechanisms underlying intersegmental locomotion in undulatory swimming.
CIRCUITY AND PATTERN GENERATION IV

FICTIVE HINDLIMB MOTOR PATTERNS EVOKED BY APPLICATION OF GLUTAMATE AGONISTS TO THE TURTLE SPINAL CORD

S. N. Currie, Dept. Neuroscience, Univ. of California, Riverside, CA 92521.

In turtles, it has been shown that both NMDA and non-NMDA type glutamate receptors contribute to cutaneous sensory processing in the scratch reflex pathway (Currie and Stein, 1990). In the present study, we tested the ability of NMDA (20-100 μM) and AMPA (2-10 μM) to evoke fictive hindlimb motor output when applied exogenously to restricted regions of the turtle spinal cord. Drugs were applied onto the exposed dorsal surface of 2-mm adjacent spinal cord segments near and within the anterior hindlimb enlargement. These segments receive cutaneous afferents from the pocket scratch and friction receptor fields (Martin and Stein, 1990) and contain both the sensory inputs of the scratch motor pattern generator (Rugiero and Crowe, 1984; Martin and Stein, 1989). Motor output was recorded unitarily from 3-5 hindlimb muscle nerves. Both NMDA and AMPA elicited coordinated burst motor discharge in all recorded hindlimb nerves. These chemically evoked motor patterns exhibit rhythmic alternation between hip flexor (VF-HP) and hip extensor (HR-AF) motor neuron activity, the timing of knee extensor activity within the hip flexor-extensor cycle is similar to that of sensory-evoked pocket scratch motor patterns. These chemically evoked motor patterns interact strongly with cutaneous-evoked reflexes. Stimulation of flexion reflexes on the ipsilateral or contralateral side produces phase-dependent resets of the chemically evoked rhythm. Stimulation within a scratch receptive field can reset the rhythm and in some cases, increase burst frequency for several cycles. Supported by NSF Grant IBN-9308804 to S.N.C.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994

THURSDAY PM
652.19
RHYTHMIC OUTPUT OF EMBRYONIC SPINAL NETWORKS IN CULTURE IS INDUCED BY DISINHIBITION BUT NOT BY INCREASED EXCITATION J. Stelzl*, Institute of Physiology, Biothiplatz 5 03122 Bern, Switzerland

Simple locomotor patterns are based on rhythmic output of local spinal networks. In order to investigate the minimal structure and the formation of such networks, the spontaneous output of cultured slices of the embryonic rat spinal cord was studied. This was done by coculturing the slices with skeletal muscle fibres and recording the patterns of spontaneous muscle contractions using a simple optical device. Neuron-driven muscle contractions were distinguished from autocontractions of the muscle fibres by their typical patterns and by pharmacological tools.

Roughly 20% of the cultures showed spontaneous neurondriven muscle contractions, most of them with random patterns. In all of these cultures rhythmic patterns of muscle contractions were induced by classical pharmacological disinhibition of the spinal networks (strychnine, bicuculline or both) and antagonism of glutamatergic (i.e. GABA B receptor) or substances interfering with cholinergic, rhabdolaminergic or peptidergic pathways had no effect on rhythmic activity patterns, suggesting that these pathways were not critical for rhythmmogenesis.

On the other hand rhythmic activity was not evoked by NMDA, glutamate or the glutamate uptake blocker dihydrokainate. NMDA in the absence of magnesium increased the rate of spontaneous activity without inducing rhythmic activity. Spontaneous activity completely ceased in presence of the glutamatergant CNQX.

These findings suggest that rhythmic output patterns arise in glutamatergic spinal networks and that they are induced by reducing inhibitory transmission within these networks but not by increasing excitatory transmission.

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652.20
NMDA-INDUCED OSCILLATIONS IN SPINAL MOTONEURONES BECOME MASKED BY INHIBITION DURING DEVELOPMENT M. S. Rios, J. Pedotti*. Department of Biology, Yale University, New Haven, CT 06511

Responses were recorded from ventral roots (VR) in isolated hindlimb frog and tadpole spinal cord (SOC). Intracellular recordings were simultaneously made from identified lumbar MNs. Tadpole swimming was recorded with a video camera. Application of NMDA (50 µM) evoked periodic bursting of VR activity in tadpole but not in frogs (5-200 µM). However, after strychnine (20-30 µM) application, synchronized periodic bursts were also initiated in the frog VRs. These persisted after strychnine removal and were coincident with periodic oscillations measured intracellularly in the motoneurones (MN). Thus, this periodic bursting behavior reflects a periodic oscillation of MN membrane potential. In the adult frog this behavior is initiated only after removal of inhibition. The periodic (-1Hz) tadpole VR bursts occur at the same frequency as the tadpole tail moves during swimming. Oscillations in the frog were much slower (>10s/cycle). Oscillations of tadpole and frog MNs have the same properties: they require the presence of physiological Mg+2 concentration (1mM), they are blocked by APV (100 µM) but not affected by CNQX (10-15 µM), and they are resistant to TTX (3µM).

These data provide evidence for the existence of a central pattern generator that is active in the tadpole and becomes inhibited during metamorphosis as the locomotor behavior changes from rhythmic to episodic.

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INVERTEBRATE MOTOR FUNCTION

653.1

Sensory inputs from the distal segments of legs have been shown to modulate walking in many animals. We report activities of sense organs of the distal (tarsal) segments of the cockroach hind leg in restrained and freely walking animals. In restrained preparations, neurographic wires implanted adjacent to sensory nerves in the first tarsal segment recorded afferent responses to the position and movements of the distal tarsal joint. We have also morphologically identified a chondrotalan organ proximal to the joint and ablation studies strongly suggest that it is the source of these activities. Recordings in freely walking animals show peak actives at the onset and termination of stance phase. These discharges occur at the time that the claw is engaged and then disengaged when walking or climbing over rough surfaces. We have also shown that electrical stimulation of afferents through the recording electrodes reflexly excites the flexor muscle. We propose that the discharge of the organ signals disengagement of the claw from the substrate, reflexly exciting the flexor muscle at the initiation of swing.

Support by ONR grant N00014-93-1-0088 and the Whitehall Foundation

653.3

In order to better understand the role of peripheral properties in cockroach walking, we have been developing biomechanical models of the insect. We have constructed a passive musculoskeletal model of the femorotibial (FT) joint of the metathoracic leg. This model suggests that passive properties (i.e. passive muscle tension and damping of the FT joint) play an important role in this joint during the swing phase of walking. Indeed, the initial swing movement can be generated entirely by passive forces. The FT joint model is currently being extended to incorporate active properties. In addition, we have developed a full body dynamic model of the free walking insect with 30 articulated degrees of freedom. This model is being used to estimate joint torques and ground reaction forces from kinematic data.

We have also constructed a leg robot with 18 active degrees of freedom. The purpose of this robot is to investigate the application of biological control principles to robotics. A generalization of leg coordination mechanisms derived from stick insect walking are used to control the robot's leg movements. These degrees of freedom are achieved using a scheme based on the equilibrium point hypothesis. Force feedback permits the robot to comply to uneven surfaces. An implementation of the elevator reflex allows the robot to step over obstacles. The robot can walk in a continuum of gaits, turn and negotiate irregular terrain.

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653.4
CHANGES IN A PROPRIOCEPTIVE PATHWAY DURING MATURATION OF LOCOMOTOR RHYTHMicity, J.R. Gray& J.M. Robertson, Dept. Biology, Queen's University, Kingston, Ontario, Canada, K7L-3N6.

The flight system of the locust is a well established model for studying the role of proprioceptive input in motor pattern generation. Gradual hemimetabolous development of this insect makes it ideal for examining maturation of preexisting circuitry as well as potential changes inafferent input.

During maturation of the flight system the wingbeat frequency increases from 13 Hz at 1-2 days after imaginal ecdysis to approximately 23 Hz 14 days later. We examined the morphology of a single cell proprioceptor, the wing hinge stretch receptor axon displays heteromorphous growth. Further, within the mesothoracic branch there is an increase in the number of axonal swellings and the branch grows positively allometrically relative to the ganglion. Artificial excitation of the stretch receptor with patterns of stimuli that correspond to increased afferent activity causes all stages of maturation produced increased activity of presynaptic flight interneurons.

These results indicate that changes in the stretch receptor may alter its effect on the operation of the flight motor and suggest that proprioceptive influences, in part, motor pattern development.

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PSIAC MODULATION OF WING HINGE MECHANICS BY THE FIRST BASAL MUSCLE OF THE BLOW FLY CALLIPHORA M. S., T* and M. H. Dickinson, Department of Organismal Biology and Anatomy, Arizona State University, Tempe, Arizona 85287.

Among the 17 direct steering muscles in flies, the First Basal (B1) has been implicated as the muscle most likely to control the timing of ventral wing pronation (ventral flip). Because of its large aerodynamic forces associated with the ventral flip, rapid and precise modulation of the B1 may be crucial for flight control. In the blowfly Calliphora vicina, the B1 fires an action potential and undergoes a cycle of length oscillation each and every wing beat at 15 Hz. Under conditions stimulating flight we previously found that the B1 may function as a spring, with properties of dynamic stiffness and mechanical energy dissipation unique to the other flight muscles. This dynamic stiffness of the B1 could control the extent of oscillations of the basal apodeme, which forms the mechanical linkage of the B1 to the wing hinge.

To test whether that rapid phase change in B1 activation can plausibly modulate oscillations of the basal apodeme, we measured B1 muscle length changes while making extracellular recordings of B1 action potentials. By measuring the deflection of a laser beam reflected from a small mirror mounted on the external portion of the basal apodeme, we could non-invasively measure oscillations of the basal apodeme and the corresponding length changes of the B1 during idled flight.

We found that changes in the interval between action potentials corresponded to increases in the maximum extent of stretch in the B1 and the extent of posterior deflection of the basal apodeme. Changes in the amplitude of basalar oscillations occurred within one cycle of changes in action potential timing. This rapid mechanical response suggests that the B1 can modulate wing kinematics on a cycle by cycle basis, even though wing beat frequency stimulation of the B1 under in situ conditions causes near-complete tetanus.

THE ROLE OF HALTERE AFFERENTS IN THE ACTIVITY OF A STEERING MUSCLE IN THE BLOWFLY, CALLIPHORA VICINA. A. Fyazuddin*, W. P. Chan, R. E. Jordan and M. H. Dickinson, Department of Organismal Biology & Anatomy, Univ. of Chicago, Chicago, IL 60637.

The large direct synchronous basal muscles in the blowfly are active during turning maneuvers and are thought to control the steering forces of the wings. Of these muscles, the first basal (B1), is unique in that it fires one spike each wing beat, at a precise phase of the stroke cycle. We have been investigating whether the campaniform fields at the base of the haltere provide the wing beat-synchronous feedback required for this sharp phase shift. Both mechanical oscillation of the haltere and electrical stimulation of the haltere nerve cause the B1 motor neuron (MNBI) to fire a phase-locked action potential. Furthermore, MNBI can follow stimulation frequencies up to and exceeding the normal wing beat frequency (150 Hz), suggesting that the halteres may be sufficient in establishing the phase-locked firing pattern of B1 during flight. We have begun to characterize the physiology of the connections between MNBI and haltere afferents by recording intracellularly recorded motor neuron while mechanically stimulating identified fields of campaniform sensilla on the base of the haltere. Stimulation of two large dorsal fields (df2 and df3), produces short-latency (<1ms) EPSPs in MNBI. Stimulation of the third dorsal field (df1) has no effect on MNBI activity. Campaniform neurons from both df2 and df3, visualized with biotinylated dextran, project to the meso-diencephalic neuropile near the site of origin of the MNBI axon. In contrast, neurons originating from df1 terminate in a different region, consistent with their failure to elicit a short-latency physiological response in MNBI. In other insect species, the strength of sensory-motor connections can be modulated by neurotransmitters such as octopamine (OA). In the blowfly, DA also enhances the synaptic efficacy of the connections between the haltere and MNBI.

THE STRUCTURE OF THE FLUID WAKE GENERATED BY A FLYING FRUIT FLY, DROSOPHILA MELANOGASTER. M. H. Dickinson, Department of Organismal Biology & Anatomy, University of Chicago, Chicago, IL 60637.

The flight of an important model system is not yet understood. In this study, we characterize aerodynamics of flight behavior in flies, I have visualized the flow using biotinylated dextran and biotinylated dextran. Flow was visualized using Lycopodium spores illuminated with laser sheets (670 nm) and imaged using a cooled CCD camera. The capture of images could be synchronized with the beat cycle following fixed delay. The generation of phase-reconstruction movies showing the change in flow through the wing stoke.

The flow visualizations reveal that Drosophila generate an inviscid single heart-shaped vortex tube during each wing stroke. The dorsal portion of each vortex ring is projected from the wing while the ventral portion of the ring is attached medially to the thorax. It is at this time that the vortex ring attaches completely from the wings and is shed into the wake. In contrast, no vortex structures are generated during the upstroke. The vortex rings generated by successive downstrokes coalesce to form a vortex tube directed posteriorly, roughly normal to the wing stroke plane. The fluid velocity within the central region of the vortex tube is high, instantaneously reaching values nearly ten times that of the free stream velocity.

The rapid shedding of vorticity during the ventral flip correlates with a large transient in the production of lift measured using laser interferometry. The rapid ventral flip shedding may provide an important means for the control of steering forces during flight.


As part of our ongoing study of metamorphosis of the frontal ganglion (FG) of Manduca, we have examined its function in the adult moth. We have found that the FG innervates the muscles of the moth's cibarial pump. These include dilators which expand the pump chamber, as well as compressors which reduce the pump chamber. During feeding the FG is involved in activating the cibarial pump muscles during feeding. The primary component consists of a relatively long burst of activity immediately followed by a brief burst of the pump compressors. The cibarial pump also plays a critical role in the expansion of the moth’s wings following its emergence from the pupal cuticle. During wing expansion, the cibarial pump is activated and the animal swallows air, using a motor pattern that is similar to that illustrated during feeding.

Air-swallowing is apparently required for proper neuromuscular function in the abdomen. Animals from which the FG was removed at the onset of metamorphosis develop normally into adults and successfully emerge from the pupal cuticle, but they fail to expand their wings and their abdomens are flattened. Feedback for turning off air-swallowing apparently requires connections between the FG and brain, as in animals in which these were bilaterally cut at the end of the larval stage expanded their wings normally but exhibited distended or burst abdomens by 24 hrs after adult emergence.

MOTOR NEURONS INVOLVED IN A SEGMENTALLY RESTRICTED REFLEX: VENTRAL FLAP AXONASIS IN PUPAL MANDUCA Sexta. C. J. Miles and R. Booker. Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

Stimulation of sensory neurons innervating hairs in the gin traps on the abdomen of pupal Manduca sexta evokes a rapid bending of the abdomen that is restricted to three posterior segments. Electrical stimulation of the gin trap sensory nerve in an isolated abdominal nerve cord evokes characteristic motor neuron activity in every abdominal segment. To determine if the segmentally distributed motor activity also occurred in intact animals and how it contributed to the segmentally restricted reflex, we measured the reflex responses of the sensory hairs in intact animals. We used reflex evoked responses as were recorded as electromyograms synchronized with video recordings of the behavior. Reflex behavior was compared with the segmental patterns of motor activity to determine if there was activity in many segments when the movement was restricted to one or two, or three segments. Coordinated motor activity was evoked by sensory nerves in response to stimulation of any of the gin traps, even when movement was restricted to one segment. Electromyograms of gin trap muscle activity correlated well with the intracellular and extracellular recordings of motor neuron activity obtained from isolated abdominal nerve cords and semi-intact preparations. These findings show that the neural circuit underlying the bending reflex is distributed throughout the abdominal nerve cord. This network generates a complex yet coordinated motor pattern with muscular activity in every abdominal segment whose concerted action produces a deceptively simple and localized bending reflex. Supported by NIH fellowships NS 07309 to W.C.L.
653.13 PERIPHERAL MODULATION OF A CENTRAL SYNAPSE BETWEEN IDENTIFIED MOTORNEURONS IN THE MEDITERRANEAN GOLLIONG OF THE LOCUST. T. Jellens & W.J. Heits*; Gatty Marine Lab., St. Andrews University, Fife, KY16 9TD, U.K. The fast extensor tibiae (FETi) motorneuron of the hind leg of locusts, which innervates the muscle providing the propulsive force for the jump and kick, also establishes excitatory synaptic connections with the fast flexor tibiae (FFTi) motorneuron. These central synaptic connections, which are apparently unique among insect motorneurons, contribute to the co-activation of the FETi and FFTi motorneurons during the preparation for a kick or jump. Simultaneous intracellular recordings from the FETi and FFTi motorneurons, while evoking ortho- and antidromic FETi spikes, reveal that the amplitude and duration of the central PSPs in the FFTi motorneurons are modulated by leg position: during leg flexion the PSPs are larger than during extension. This is in part due to sensory feedback of information in the ascidian muscle stimulating the central FETi-FFTi synaptic component. However, when extensor tension is abolished by dye-mediated laser photo-oxidation of FETi, the amplitude of the central PSPs is still modulated by leg position. The photo-oxidation technique allows specific de-innervation of the extensor muscle, without affecting any of the other structures, and the innervation can swiftly be restored via an electronic "sensory bypass" from the FETi soma to the extensor muscle. In the locust, hind leg position is mainly monitored by the chordotonal organs located in the distal femur. Manipulation (alternating stretching and relaxing) of the tendon of this sense organ, while keeping the leg in fixed position, accounts for most of the PSP amplitude modulation. A relatively enhanced PSP in the FETi with leg flexion makes sense since leg flexion is a prepossess to go into the co-activation phase. The site and mechanism of the modulation (e.g. pre-synaptic FETi spine amplitude, input resistance of the FETi, presynaptic modulation of the central synapse) itself is currently being investigated.

653.14 NEURAL ELEMENTS OF GRASSHOPPERS SUPPORTING SEXUALLY DIMORPHIC BEHAVIOR. K.J. Thompson and J.L. Roosevelt, Dept. of Biology, Agnes Scott College, Decatur, GA 30030. In a continuing study of the neural basis of oviposition behavior in grasshoppers, motor neurons (MNs) and dorsal unpaired median neurons (DUMs) of the terminal ganglia of male have been identified by backfilling lateral nerves with CoCl2. This information is being compared to the activity of known courtship behavior. In addition, the differences in MN and DUM pattern have been observed in males and females. Two hypotheses for how the nervous system produces sexual dimorphism in behavior are being tested. 1) Male-deficient hypothesis, which predicts that the male nervous system is missing female-specific output neurons (males do not lay eggs) and 2) Male-specific hypothesis, which predicts that the male contains neurons homologous to ovipositor neurons but involved in other functions. Four clusters of ovipositor MNs have been previously identified in females. Two of these clusters are present in the male DUMs have axons in different branches of the 8th terminal nerve of the terminal abdominal ganglion. One cluster, the protracor, is a group of 3 MNs with cell bodies contralateral to the file nerve and the second cluster, the closer, is a group of 3 MNs with cell bodies in the anterior ganglion. Backfills of the terminal nerve in males produced a similar pattern of MNs, in terms of numbers of cell bodies, cell body positions, and distinctive axonal trajectories. A single DUM neuron, located in the terminal abdominal ganglion, fills from the terminal nerve in both sexes. Thus, for two of the four groups of efferent neurons that have been identified, limited gender differences in MN and DUM complement have been found, despite differences in male and female behavior, and differences in the size and shape of the terminal ganglion. Supported by Whitetail grant 8ABS-01.

653.15 DYNAMICS OF A POSTURAL REFLEX IN HERMIT CRAB STUDIED WITH SINUSOID AND NOISE VIBRATION. W.D. Chappel*; Dept. Physiology and Neurobiology, Univ. of Connecticut, Storrs, CT 06269. A systems model of a phasic stretch reflex, important in the postural support of its shell by the underlying muscle. Pogoins pellucidae, has been developed from experiments using simultaneous recording of muscle force, length, and spine frequencies of pairs of motorneurons during ramp stretch and release of the right fourth segment of the abdominal ventral superficial muscles. (Chappe1993). A prediction of this model is that the reflex is important primarily in increasing abdominal stiffness in response to sustained rather than transient stimulation. Sinusoidal and broadband noise loading lasting for 2 sec was used to vibration the muscle. Reflex activation of the motorneurons occurred at on the 80% of optimum 20 Hz and frequencies above 2.5 Hz. A rapid onset, peak, frequency of this reflex is consistent with the model for the damped second order system. This result indicates that the motorneurons increased muscle stiffness and the reflex input appears to be increased, but did not alter its dynamics. The form of the transfer function that best described the reflex was that of a high pass frequency of 10 Hz, with a deviation of the isolated, electrically stimulated muscle. The model's prediction that the muscle reflex could increase muscle stiffness during sustained vibration was confirmed. However, in this isolated abdominal preparation, the reflex did not compensate for vibrations in muscle stiffness with stimulus amplitude, and, thus, did not regulate muscle stiffness.

653.16 DEVELOPMENT OF STOMATOGASTRIC MEDIATED MOTOR PROGRAMS IN THE AMERICAN LOBSTER. Kari Lavalli, Joseph Ayres*; Marine Science Center, Northeastern Univ., East Point, Nahant, MA 01908. Feeding and stomatogastric mediated behavior are composed three phases in all larval stages: an ingestion phase a relatively quiescent phase and a periodic grinding phase. We have demonstrated three behavioral stages of development of stomatogastric behavior. The first stage is limited to Stage I and II larvae and consists of: a) a fast peristaltic component which occurs only during ingestion; b) a slow "cardiac sac" rhythm which begins several minutes after the cessation of feeding and continues on until the gut is empty. This rhythm is the only present for the first four post-feeding and may serve to mix ingested food with digestive juice. It then allows a 3) the "gastric" rhythm resulting from a constriction of the lateral walls of the cardiac sac and a forceful anterior movement of the entire cardiac sac. No separate pyloric rhythm is formed in either Stage I or II larvae. The second behavioral stage is observed only in Stage III larvae and adds a medial gastric component due to elaboration and participation of the medial tooth. The third behavioral stage is first observed in Stage IV postlarvae and demonstrates an excellent replica of the adult pattern with several forms of modulation. Here, separate sacs, gastric and pyloric are present. After Stage IV have been able to identify different modes of operation of the gastric milk, particularly that of "cut and grind" and "superate." In addition several modes of modulation of the movements are present in all three stages. Supported by NSF Grant IIB-912224.
653.17 ENHANCEMENT OF NEUROMUSCULAR EFFICACY BY FLRF PEPTIDES IN THE CRAB, Cancer borealis. (J.C. Corpse, Ryan* and E.Stark. Ch. for Complex Systems, Brandeis University, Waltham, MA 02254)

Muscles play an essential role in re-shaping the activities of the gastric and pyloric water patterns of the stomatogastric ganglion (STG) of Cancer borealis (Winfield et al., 1993). This includes: enhancement of the pyloric rhythm frequency, activation of segment-specific pyloric rhythm motor patterns, and activation of oscillatory properties in the Dorsal Gastric (DG) neuron. We now use neuromuscular preparations to determine if FLRF agonist peptides increase neuromuscular junctional properties.

We found that both POP-FLRFamide and TPR-FLRFamide increase nerve-evoked contractile responses. Extrinsic Junctional Potentials (EJP’s) on DG-innervated muscles, gastric muscle and pyloric muscle were both enhanced by these peptides at concentrations ranging from 10^-10 M to 10^-4 M. POP-FLRFamide produced a 100% increase and TPR-FLRFamide a 90% increase in contraction. These effects are dose-dependent.

Muscle contractions are also shown to be modulated by neuromuscular junctional properties. Modulation of the NMj by itself will amplify insecticidal inputs, or will ensure that the changes in the muscle rhythms will be translated into changes in motor outputs. This will have different behavioral consequences depending on the physiological context where modulation of the NMj takes place.

Supported by NS17813.

653.19 STATIC AND DYNAMIC-RADULA/ODONTOPHORE KINEMATIC MODELS OF THE BUCCAL MASS OF APLYSIA CALIFORNICA. B.F. Drcsler,1 H.L. Cad,2,3 and E. Cragg.2 Departments of Biology, 1Biomedical Engineering, and 3Neuroscience, Case Western Reserve University, Cleveland, Ohio 44106, U.S.A.

A computer kinetic model of the buccal mass of Aplysia californica (Neustadter, 1992) was revised to more accurately reflect the true shapes and dimensions of buccal mass components in vivo. The Neustadter model represents the radula/odontophore as a sphere, the I1/12 muscles as 6 toruses, and the I2 muscle as a posterior band connecting the dorsal and ventral aspects of the I1/12 torus; a ventral hinge attaches the I1/12 and I3/12 elements to the odontophore. Protraction/retraction is accomplished by forward/backward rotation of the sphere, and the I1/12 toruses expand/constrict to continuously change the surface of the sphere.

Buccal muscle shapes from transilluminated feeding slugs and from models were classified as rest, protraction, or retraction. Actual measurements of the shapes were used to develop a model of buccal mass in the "space shape". In vivo, shapes classified as rest/protraction/retraction cluster together in distinct regions of the space shape plots. Homogeneous model geometry only roughly equivalent to those observed in vivo, and the shape space plots did not match. To improve the model, two different odontophore shapes (#1 and #2) derived from dissection were substituted for the odontophore of the Neustadter model. These shapes were asymmetric in mid-segmental section, though assumed to be circular in cross section when in contact with the I1/12 torus. The model was rewritten to account for arbitrary, asymmetric shapes. Shape #1 gave accurate buccal mass retraction shapes, but poor rest/ protection shapes. Shape #2 gave accurate rest/protraction shapes, but poor retraction shapes. In both cases, the space shape plots, while improved compared to the Neustadter model, did not match those from in vivo.

Since no single odontophore shape gives correct buccal mass shapes over the entire range, we hypothesized that the odontophore shape changes dynamically as it moves. We are currently analyzing videotapes of transilluminated feeding slugs to generate a gallery of possible morphologies to better understand the surrounding space. At the 13/1 elements to be fit to arbitrary, non-circular cross sections. NIH PPG HL-25830-11A1.


Identified cholinergic motor neurons (Ms) and serotonergic modulatory neurons (Pps) in the pedal ganglia of A. brasiliensis innervate peripheral parapodial muscle and fire rhythmically during swimming locomotion. POP-cell firing increases the amplitude of MS action potentials (AMPs) in the corresponding junctional potentials (EJPs) in muscle fibers and increases their contraction amplitude and relaxation rates. This study was designed to determine whether part of the facilitatory effect of serotonin (5-HT) is due to presynaptic action potential (PAP) inhibition. This study was designed to determine whether part of the facilitatory effect of serotonin (5-HT) is due to presynaptic action potential (PAP) inhibition. This is a critical question in control of muscle with modulatory inputs, because sufficient activation of MSs could facilitate movement by enhancing presynaptic inhibition. 5-HT has complex effects on AMPs in muscle fibers, which may be enhanced or decreased depending on its concentration. At low concentrations of 5-HT, the AMPs were enhanced, whereas high concentrations suppressed AMPs.

653.20 TEMPERATURE SENSITIVITY OF THE GIANT MOTOR SYNAPESE AND TAILFILIP BEHAVIOR IN CRAYFISH. M.F. Burgess, F. Issa, D.H. Edwards and W.J. Butler. Gatty Marine Laboratory, School of Biological and Medical Sciences, University of St. Andrews, Fife, KY16 9LB, Scotland, and Department of Biology, Georgia State University, Atlanta, GA 30303-4010.

The rectifying electrical giant motor synapse (GMS) between the giant fibers (GFs) and motor giant neuron (MGn) has been previously shown to have an extremely temperature sensitivity. The large difference between the Q10 of the GMS (near 11) and that of the active membrane in the GF (generally 3) pointed us to examine whether effective transmission would fail at temperatures away from room temperature and so prevent GF-mediated tailflips. We found that transmission was reliable at cold temperatures down to 4°C as both the presynaptic GF and postsynaptic (MGn) spikes and the MGn EPSPs broadened considerably. Transmission failed at warm temperatures, usually between 25°C and 30°C, as the duration of the presynaptic spike shortened. Compartmental models of the circuit indicate that transmission fails at high temperatures when the GF spike is too brief to inject enough depolarizing current into the MGn to reach threshold. Transmission is maintained in the cold because presynaptic spike broadening compensates for the slowing of the GMS conductance change. GF stimulation in freely behaving animals evokes tailflips that fail between 25°C and 30°C, and are gradually reduced in amplitude between 15° and 5° C. Recordings in an isolated abdomen indicate that tailflip failure at high temperatures results from transmission failures at the GMS, whereas tailflip reduction in the cold appears to result from slowing of neuromuscular transmission, excitation-contraction coupling, or constriction of the fast flexor muscle that produces the tailflip.

653.22 A POPULATION OF CEREBRAL INTERNEURONS MEDIATE THE COORDINATION OF VASCULAR RESPONSES INVOLVING FEEDING BEHAVIOR OF APYLSIA. Y. Yin, K.I. Weiss and J. Kimmel*. Center for Neurobiology and Behavior. Columbia University, 722 W. 168 St. New York, NY 10032 and Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029.

A population of neurons (CC neurons) were identified in the cerebral ganglion C cluster. They were found to affect various parts of the body wall as well as cardiovascular and syrinx parts. CC neurons produce mono or polysynaptic effects on various cerebral, pedal, plural and abdominal ganglion neurons. The CC neurons receive synaptic inputs when the lip is touched with food or during buccal mass movements. They have mutually excitatory or inhibitory interactions with the feeding command-like neuron C-PF1. CPF1, which receives presynaptic input from both the cardiac interneurons and the chemosensory neuron (PAS), is excited by touch of the lip by either ingestion or by touch of the buccal mass retraction. C7C, which monosynaptically excites abdominal vasomotor neurons (LbVc cells), is excited during buccal mass protrusion and inhibited by a brief touch of the lip. CC6, which monosynaptically inhibits the ipsilateral arterial motor neuron, could be excited by either the feeding of the C-PF1 and is held by touch to the ipsilateral lip. C4C4, which excites a neuron that shortens the buccal artery, is excited during buccal mass protrusion and by a touch to the lip. C3C3, which monosynaptically excites abdominal heart excitatory neurons (RBm cells), increases its firing rate when the lip is touched with food. C2C2, monosynaptically excites the abdominal L2 neurons, while firing a C2C2 burst. Hyperpoliarization of specific CC neurons revealed that they mediate all or most of the synaptic effects of buccal mass movements and lip stimulation on cardiovascular motor neurons. Our results indicate that a small population of the cerebral interneurons orchestrates a complex pattern of cardiovascular responses during feeding behavior.

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653.23
STRETCH-ACTIVATION OF THE ACCESSORY RADULA OPENER MUSCLES OF APLYSIA.
The accessory radula opener musculature of Aplysia is comprised of seven strap-like muscles, one pair and one single), named I7, 18, 19, and 110, innervated by a common cholinergic buccal motor neuron, 848. The muscles originate from a muscular sheath covering the collydostyle at the base of the radula sac and become stretched as the accessory radula closes muscles contract. Even when denervated, the muscles will contract rhythmically if subjected to a constant stretch. Intracellular recordings from muscle fibers of 17 reveal that stretching the muscle induces a depolarization which can cause a slow increase in tension followed by discrete contractions accompanying muscle fiber spikes. The stretch-activated depolarization appears to be sodium-dependent since it is reversibly abolished in sodium-free artificial seawater. Nifedipine, at concentrations of 10^-6 and 10^-7M, can abolish the spikes but not the stretch-activated depolarization. The stretch-activated depolarization may augment the depolarization of the muscle fibers by the motor neuron-elicited excitatory junction potentials to promote a greater contraction. Indeed we found that motor neuron-driven contractions of 17 initiated from a constant length decreased as the load increased, but if the length was permitted to increase, the amplitude of contraction increased. The stretch-activated depolarization may represent a peripheral mechanism to ensure that the muscle is optimally responsive to the mechanical demands of the system.

653.24
THE WHOLE-BODY SHORTENING REFLEX OF THE LEECH: MOTOR PATTERN, SENSORY BASIS, AND INTERNEURONAL PATHWAYS. B.K. Shaw* and W.B. Krustan, Jr. Dept. of Biology, Univ. of California at San Diego, La Jolla, CA 92039-0357.
The leech whole-body shortening reflex consists of a rapid, synchronous contraction of the whole body elicited by a strong mechanical stimulus to the anterior of the animal. We have studied this reflex in semi-intact and isolated nerve cord preparations. The motor pattern consists of an alternating bilateral co-contraction of the longitudinal muscles (DE and VE respectively), as well as the LC cell, a motor neuron innervating both dorsal and ventral longitudinal muscles. The motor pattern has phasic and tonic components: the LC cell is transiently activated, while cell 3 (a representative DE) shows a more sustained activation. Stimulation that produce shortening activate the T (touch), P (pressure), and sometimes the N (nociceptive) types of mechanosensory neuron. Stimulation single-sensory neurons did not cause the behavior; if appears that a critical number must be activated to yield shortening. The S cell network, which makes up a fast conducting pathway running through the leech nerve cord, is active during shortening and accounts for the shortest-latency excitation of the LC cell. However, the S cell network is not sufficient by itself to account for the reflex; firing the S cells at physiological rates with an intracellular electrode causes just a weak excitation of LC cells and little to no behavior. Recordings from the nerve cord connective during shortening reveal fast-conducting spikes besides those of the S cell; these spikes are correlated with motor neuron PSPs. This suggests that the S cell network and other pathways operate in parallel to produce the reflex. Supported by an NSF Predoctoral Fellowship (BKS), NIH training grant (BKS) and NIH research grant MH44396 (WKB).

653.25
MAPPING MOTOR NEURON ACTIVITY TO OVERT BEHAVIOUR IN THE LEECH: A BIOMECHANICAL MODEL OF THE LEECH HYDROSKELETON. R.A. Wilson*, R.A. Skerretzky, J.K. Myers, S. Blackwood, R. Rakia, W.B. Krustan, Jr. 1st Dept. of Biology & Institute for Biomedical Engineering, UCSF, La Jolla, CA 92039-0357.
The leech has proved a good system in which to determine how neuronal elements generate motor neuron activity. However, little is known as to how these neuronal patterns coordinate the behaviour of the animal as a whole. We have undertaken a study to determine how motor neuron activity interacts with the biomechanical properties of the leech hydroskeloton to produce overt behaviours. A model was developed to predict the shape of a leech for a given spatial pattern of motor neuron activity. The model was based on the following experimental data: the dimensions of the body at rest or following intrinsic or extrinsic contractions, the passive length-tension properties of the muscles in the body wall and the active tension produced by activating single identified motor neurons at different muscle lengths and firing frequencies. We made the model on the assumption that (a) the volume of each segment remains constant and (b) when as a steady-state the shape of the leech such that the potential energy of the whole body is minimized. The potential energy for the whole body was calculated as the sum of that for each muscle, each in turn was calculated as the integral of the corresponding active and passive tension over the length of the muscle. A key test of the model has been a comparison between predicted and observed pressures recorded in the gut during the extremes of four very different behaviours: crawling, swimming, shortening and feeding. The model as it stands provides new insight into the biomechanical properties of the hydroskeloton and how these properties may be adapted for behaviour. We intend to make the model more realistic by including neuronal, muscle and fluid dynamics. Supported by NSF Research Grant IBN9222039.

654.1
We are interested in the expression and regulation of fiber-type-specific contractile protein isoforms in developing fast skeletal muscle fibers. This current study focuses on the early post-natal expression of a Trifast-Lace chimeric transgene that is driven by an avian Trifast promoter. Unlike the endogenous Trifast gene, this chimeric transgene is differentially expressed in the fast muscle fibers of adult mouse, i.e. IIB >> IIA. In the neonatal transgene mouse, this differential expression is not observed at birth, but emerges during the first week of postnatal life. These results suggest that transcriptional mechanisms present in IIB fibers may be distinct from those operating in IIA fibers. Because its concentration peaks during the first post-natal week, thyroid hormone may subserve such a mechanism and act to direct the high level Trifast-Lace expression seen in IIB fibers. To investigate the role of thyroid hormone in the post-natal emergence of the differential expression pattern, muscle sections from hypothyroid (induced by 5-3-propyl-thiouracil-depletion) and untreated-control neonates will be compared and analyzed using quantitative beta-galactosidase histochemistry.

654.2
DISTRIBUTION OF MYOSIN HEAVY CHAIN-BASED INTRASFAL FIBER TYPES IN SMALL SPINDLES OF CHICKEN LEG MUSCLES AND THEIR DEVELOPMENTAL SIGNIFICANCE. A. Major*, Dept. of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294.
The myosin heavy chain (MHC) composition of intrafusal fibers in postnatal chicken leg muscle spindles containing from 1-4 intrafusal fibers was examined in sections incubated with monoclonal antibodies against slow or fast MHC. In those muscles receptors with fewer than five intrafusal fibers made a substantial portion of the spindle population. The small receptors are presumably normal spindles, except that they acquired fewer than the average number of seven intrafusal fibers. Oligofibrilar postnatal activation of sensory motor neurons innervating dorsal and ventral longitudinal muscles appears to be a determinant of the order of appearance of intrafusal fiber types, which in embryonic tissue is difficult to assess. The single fiber in postnatal multifibrillar spindles almost always had a fast MHC profile. In multifibrillar spindles there were a 60-40 fast/slow split. Spindles with three intrafusal fibers typically presented two fast and one slow fiber, while in quadriofibrilar spindles the number of fast and slow intrafusal fibers was nearly equally divided. These data support the earlier finding that the proportion of slow to fast intrafusal fibers increases gradually from the onset of spindle development until the time when 5-6 intrafusal fibers have been formed (Cell Tiss Res, 274:383, 1993). The constant fiber type composition of small postnatal spindles also suggests that the first four fibers in a spindle arise in a fast-slow-fast-slow sequence, provided there is no significant postgenesis MHC transformation.

Neural regulation of slow-tonic (S), α-cardiac (α-c), 2A and 2B myosin heavy chains (MHC) was investigated in rat muscle spindles which regenerated in the presence of sensory innervation only (DE), motor innervation only (MN) or both (MN+DE) in the absence of all innervation (DN group). Either dorsal root ganglia L3-6 were removed (N=9), ventral roots L3-6 were severed (N=10) or sciatic n. was severed (N=5) in young adult female rats. Three days later, a nerve-intact orthotopic graft was performed to regenerate new sensory axons in the ventral root. After an additional 7 days, the grafts were excised. These results indicate that the absence of sensory innervation is necessary for increased fiber type proportions in adult muscle. These observations suggest that definitive changes in fiber type composition occur in the adult muscle. (Supported by N.I.H. grants HL44923 and GM33171.)


In many human muscles aged-related partial denervation leads to muscle fiber "type grouping": a departure from the normal tendency toward random spatial distribution of muscle fiber types. Techniques for assessing muscle fiber "type grouping" have generally been based on two-dimensional models that are not applicable to geometrically complex muscles such as the thyroarytenoid. In the present study, a design-based (free of assumption of a geometrical model) second, stereological technique (Kroustup et al., J. Microsc. 149:135) has been used to quantify age-related muscle fiber "type grouping" in the human thyroarytenoid.

In humans the thyroarytenoid muscle plays crucial roles in protecting the airway and in vocalization, and age-related changes in this motor system are of concern. Samples were obtained from male and female autopsies (cases 49-97 years; n=20). The results indicate a significant (P=.032) age-related increase in the tendency for muscle fibers to be nonrandomly grouped in clusters with fibers of the same type (based on expression of or lack of expression of fast myosin isoform). Since this phenomenon is the result of reinervation of denervated fibers by collateral sprouting of a common axon, the muscle fibers in these clusters tend to alter their myosin isoform expression in response to the influence of the new motor neuron. This result therefore indicates an age-related partial denervation followed by regeneration in the human thyroarytenoid muscle, which is consistent with a hypothesis of a shift toward neuron cell death in this system. (Supported by NIH grant AG0918603)

654.5 NEUROMUSCULAR JUNCTION MORPHOLOGY DURING FAST-TO-SLOW MUSCLE TRANSFORMATION. T. Somasekhar*, R.H. Nordlander and P.R. Reiser, Dept. Oral Biology, Ohio State Univ., Columbus, OH 43210.

Chronic low frequency stimulation results in transformation of muscle from fast to slow-twitch phenotype. We examined the morphology of neuromuscular junctions (NMJs) in two fast-switch rabbit muscles, extensor digitorum longus (EDL) and tibialis anterior (TA), after 3 weeks of chronic electrical stimulation at 10 Hz. These muscles were stained with rhodamine-conjugated monoclonal alpha-bungarotoxin and their NMJs analyzed with Meta-Morph software. Stimulated TA muscles showed significant reductions in mean NMJ area (30%), length (15%) and width (15%) compared to unstimulated control TA muscles (n=2 rabbits). Changes in NMJ morphology were more profound in TA than in EDL. Mean NMJ area in normal slow soleus muscle was similar to control EDL but was significantly smaller (22%) than control TA. A similar range of NMJ configurations were seen in both controls and stimulated muscles. These observations show that stimulation induces marked changes in NMJ size. Work is underway to determine whether NMJs simply shrink in size or selectively retract branches during fiber transformation. Supported by NIH AR39652 and NS18773.

654.6 IMMUNOREACTIVITY FOR THE N-METHYLDOPAMINE RECEPTOR-NACTYL-ASPARYLGLUTamate AND NAALADASE AT THE RAT NEUROMUSCULAR JUNCTION. U. V. Berger* and T. T. Coyle, Laboratory of Molecular and Developmental Neuroscience, Massachusetts General Hospital, Boston, MA 02129.

The neuropeptide N-acetyl-asparylglutamate (NAAG) is co-localized to a number of different neurotransmitter systems in mammalian central nervous system including the ventral horn cholinergic motoneurons. NAAG is released upon depolarization and catalyzed by the extracellular enzyme NAALADase to N-acetylaspartate and glutamate. Furthermore, NAAG displays partial agonist activity at NMDA-receptor glutamate receptors. The physiological function of NAAG is still uncertain, but may include a role as a neuromodulator or as a glutamate precursor. In the peripheral nervous system, NAAG-like immunoreactivity (LI) is present in axons of sensory and motor nerves while NAALADase is expressed by non-myelinating Schwann cells. The present study investigated the presence of NAAG, NAALADase and NMDA-1 receptors at the rat neuromuscular junction. Three days after sciatic nerve ligation, the peripheral nerve terminals of the glutaconelike immunoreactivity in the endplate region of the diaphragm using immunofluorescence and immunoblotting. The NMDA-1 receptor was closely associated with alpha-bungarotoxin binding to nicotinic acetylcholine receptors, suggesting that NMDA receptors are present postsynaptically on muscle cells. The presence of NAAG in motor nerve endings corroborates other recent findings of glutamate-like immunoreactivities in rat motor neurons and terminals. These results suggest that NAAG, or glutamate cleaved from NAAG by NAALADase, may be involved in vertebrate neuromuscular transmission.


Previous studies have shown 1) differences in endplate size on slow type I and fast type II muscle fibers, and 2) correlation between endplate size and muscle fiber type. The present study, the three-dimensional (3D) surface area of endplates on type I & II muscle fibers of 21-day old (D1) and adult rat diaphragms were examined using confocal microscopy. Muscle fibers were labeled with fluorescein o-bungarotoxin, and muscle fiber type was determined using an anti-fetal myosin antibody and indirect immunocytochemistry. Surface areas were calculated from computer-generated reconstructions of confocal optical sections. At D1, in spite of no difference in fiber size, surface areas of type I fiber endplates were greater than those of type II fibers. These differences were also observed in adults, even though type I II fibers grew disproportionately to type I fibers. In adults, endplate surface area was positively correlated to muscle fiber size, only within a fiber type. When normalized for fiber diameter, surface area of type I fiber endplates was significantly greater than that of type II fiber endplates, in both age groups. In type I fibers, the increase in endplate surface area was disproportionate to fiber growth, but in type II, the increase in endplate area was proportionate to fiber growth. We conclude that differences in endplate size in type I & II fibers reflect phenotypic differences unrelated to differential fiber growth. These differences in endplate size may impact synaptic efficacy. (Supported by NIH grants HL37680, HL34318 and GM02883.)

654.8 INFLUENCE OF INACTIVITY ON ENDPALTE SIZE AND NEUROMUSCULAR TRANSMISSION. H. Miyata, W.Z. Zhan, Y.S. Prakash and G.C. Sieck* Departments of Anesthesiology and Physiology, Mayo Foundation, Rochester, MN 55905.

In the rat diaphragm (DIA), we induced inactivity of the right hemidiaphragm by hemisection of the C2 spinal cord (spinal isolation, SI) or by tetorodotoxin (TTX) blockade of phrenic axonal propagation. After 2 weeks of inactivity, we examined adaptations in type I and type II fibers. We measured fiber diameters on type I and II muscle fibers, as well as susceptibility to neuromuscular transmission failure (NF). Paralysis of the right hemidiaphragm was confirmed by the absence of a twitch. EMG activity was measured in vivo by stimulating the phrenic nerve at 40 or 75 Hz in 330 ms trains repeated sec for 5 min. Direct muscle stimulation was superimposed every 15 s. The following EMG activity were classified as estimated NF. Motor endplates were labeled with fluorescein-conjugated o-bungarotoxin and muscle fiber type was determined using an anti-fetal myosin antibody. Double-labeled samples were analyzed using dual channel laser-scanning confocal microscopy to reconstruct 3D images of motor endplates. In DIAL type II fiber endplates were larger than in type II fibers. Type II fiber endplate size increased in the TTX and SI diaphragm but type I fiber endplate size was unchanged. As compared to CTL, NF at both 40 and 75 Hz was less pronounced in the SI group but more pronounced in TTX animals. We conclude that endplate adaptations to inactivity are restricted to type II fibers, but do not necessarily reflect associated changes in neuromuscular transmission. Supported by NIH grants HL34817 and HL37680.

Neuronal activity of the suprachiasmatic nucleus (SCN) is known to be influenced by two major innervations to the SCN: photic stimuli conveyed by the direct retinohypothalamic tract or indirect geniculohypothalamic tract, and the seronicergic (5-HT) neurotransmission by ascending forebrain 5-HT tract. In the present study, we examined the time-course of VIP mRNA expressions in the SCN of rats in which the two major neuronal transmissions were eliminated, by using in situ hybridization technique combined with in situ immunohistochemistry. Male Wistar rats were housed in constant darkness, and the 5-HT input to the SCN was blocked by the three days successive intraperitoneal injections of p-chlorophenylalanine methylester (300 mg/kg/day), an inhibitor of tryptophan hydroxylase. After 24 hrs of the last injection, VIP mRNA levels in the SCN were assayed by in situ hybridization, in saline treated controls, VIP mRNA levels were almost constant at any time of the day. In contrast, PCPA treatment induced the rhythm of VIP mRNA with the peak at CT 4 and trough at CT 20. These findings suggested that the removal of photic and 5-HT influence induces VIP mRNA rhythm in the SCN, and indicates that VIP mRNA is controlled also by the circadian clock.

655.2 DISTRIBUTION OF NEUROPEPTIDES IN THE HAMSTER SUPRACHIASMATIC NUCLEUS. Kazuo Sonobe*, H. D. Pagani and Benjamin Rusak. Deps. of Anatomy & Neurobiology, and Psychology, Dalhousie University, Halifax, N.S., Canada, B3H 4J4

The suprachiasmatic nucleus (SCN) of the hypothalamus are the site of the primary circadian pacemaker in mammals. The distributions of various neuropeptides have been described in the rat SCN but have not been well localized in these mammals as the hamster. Using mono- and polyclonal antibodies, we examined the distribution of immunoreactivity for calcitonin gene-related peptide (CGRP), galanin (GAL), gastrin-releasing peptide (GRP), neurokinin A, vasointestinal peptide (VIP), and vasopressin (VP) in the SCN of both cholecyste treated and untreated Syrian hamsters. Intense labeling of cell bodies, fibers and terminals was observed for GRP, PH, VIP, and VP within the SCN. In contrast, only sparse labeling of GAL and SP immunoreactive fibers and terminals was seen in the SCN, while intense labeling was present in adjacent hypothalamic areas. A small number of SP-positive cell bodies were observed in the central SCN, whereas GAL-positive cell bodies were found only in hypothalamic areas bordering the SCN region. A few CGRP-positive fibers and terminals were found in the ventral and lateral aspects of SCN, but no cell bodies stained for CGRP.

In summary, various neuropeptides show different patterns of distribution in the hamster SCN. These data suggest different functions for these peptides in circadian processes. Supported by grants from the MRC of Canada, and a postdoctoral fellowship from the MRC to HDP. K.S is an MRC Scholar.

655.3 ELECTРОPHYSIOLOGICAL EFFECTS OF IОНОPHORETICALLY APPLIED SUBSTANCE P ON HAMSTER SUPRACHIASMATIC NUCLEUS IN ВITRO. Hugh D. Pagani*, David J. Cutler, and Benjamin Rusak. Deps. of Anatomy & Neurobiology, and Psychology, Dalhousie University, Halifax, N.S., Canada, B3J 4L1

The suprachiasmatic nucleus (SCN) of the hypothalamus function as the primary circadian pacemaker in mammals. The tachykinin Substance P (SP) and tachykinin receptors have been identified in the rat SCN, but little is known about the role of SP in circadian processes of other mammals. In this study, we examined the effects of local applications of SP on the extracellularly recorded discharge rate of Syrian hamster SCN neurons maintained in an in vitro brain slice preparation. Brain slices (400-500 μm thick) were prepared from hamsters housed under a 14:10 light:dark schedule during the light phase of the cycle. SP (1 μM) or 165 mM NaCl) or vehicle, were ionophoresed as cations (20-400 nA) from a multibarrel pipette located 50-80 μm from a recording electrode (containing 2 M NaCl) situated in the SCN. When collapsed across a recording session (ZT4-ZR), both SP and vehicle increased the firing rates of 57 cells, and suppressed the firing rates of 13 cells of 146 cells tested. In contrast, control ejection of vehicle evoked responses from only 3 of 36 cells tested. A larger percentage of cells responded to SP during the middle of the projected day (62.5%) than during the middle of the projected night (25%). This data indicate that local ejections of SP can alter hamster SCN neuronal activity, particularly during the projected day. Small this raises the possibility that local release of SP from intrinsic SCN neurons or from afferent projections may play a role in the entrainment of the circadian pacemaker.

655.5 DEVELOPMENT OF GLIA AND VIP-IMMUNOREACTIVE NEURONS IN THE HAMSTER SUPRACHIASMATIC NUCLEUS (SCN). G.I. Botchkina* and L.P. Morin, Dept. Psychiatry, Stony Brook University, NY 11794

Radial glia are thought to guide neurons to their targets in development. We attempted to facilitate their compartmentalization by studying the relationships between radial glia, astrocytes and VIP-IR neurons in the SCN, site of the mammalian circadian clock.

Vimentin-IR (VIM) radial glial processes are evident as early as embryonic day 8 (ED 8), achieving their highest density in the SCN by ED 13-14. By postnatal day 5 (P5), VIM-IR radial glia in the SCN have gradually disappeared. VIP-IR identifies a set of ventral SCN neurons as early as ED 13. A VIP-IR fiber plexus is evident in the SCN by ED 15. If then progressively increases in density while extending dorsally.

The first GFAP-IR appears in glia of the ventral SCN and optic chiasm by ED 15. GFAP-IR astrocytes are evident by P0 and they progressively increase in number to adulthood. A few VIP-IR astrocytes are present in the SCN by ED 21. The first embryonic hamster circadian clock may be functional at ED 15. Thus, it is possible that astrocytes may not appear until after embryonic rhythmicity has begun. Supported by NS22168.

655.6 MUSCIMOL REDUCES PHASE DELAYS PRODUCED BY COADMINISTRATION OF Vasoactive Intestinal Peptide (VIP), Peptide Histidine Isoleucine (PHI), AND GASTRIN RELEASED Peptide (GRP) INTO THE SUPRACHIASMATIC NUCLEUS (SCN). C.P. Gillespie*, T.O. Bhubahoni, K.L. Muham, H.E. Albers, Lab of Neuroendocrinol. & Behav., Georgia State Univ., Atlanta, GA 30303

Coadministration of VIP, PHI, and GRP into the hamster SCN produces large phase delays in activity rhythms. Since GABA has been reported to be found in most neurons of the SCN, the present study examined the phase shifting effects of coadministration of the GABA, agonist, muscimol, with VIP, PHI, and GRP. Male golden hamsters were implanted with guide cannulae aimed at the SCN region and housed in constant darkness. A stable free-running rhythm of wheelrunning was established, the hamsters were microinjected at CT 12-14 with a 200nl cocktail consisting of either VIP (50ng in 50nl saline) + PHI (5ng in 50nl saline) + GRP (50ng in 50nl saline) + muscimol (150ng in 50nl saline), or VIP + PHI + GRP + 50nl saline in a counterbalanced order. Microinjection of the cocktail containing muscimol resulted in phase delays (0.23 ± 0.06hr) that were significantly smaller (p<0.001) than when the microinjection of the cocktail not containing muscimol (0.88 ± 0.13hr).

These data suggest that GABAergic activity within the SCN may be important in regulating the phase-delaying effects of VIP, PHI, and GRP. Supported by NS30002.
ROLE OF SUBSTANCE P IN MEDIAL AMYGDALOID SUPPRESSION OF PREDATORY ATTACK BEHAVIOR IN THE CAT.
Y. C. Han*, M. B. Shaikh and A. Siegel, Laboratory of Limbic System & Behavior, Department of Neurosciences, New Jersey Medical School and Graduate School of Biomedical Sciences, UMDNJ, Newark, New Jersey 07103.

In the present study, we tested the hypothesis that (1) the medial amygdala (ME) suppresses quick biting "predatory" attack behavior (QBA), and (2) substance P (SP) is utilized as a neurotransmitter in the pathway from the ME to the hypothalamus that mediates progression of QBA. Phase I: QBA in ME lateral hypothalamic site (LH) were observed to be optioned by electrical stimulation. Response latencies for QBA were significantly increased (p < 0.05) after additional stimulation of both sites. Phase II: microinjections of the NK, antagonist, CP, 96,345 (0.5, 0.5 and 2.5 mg/kg) were placed into the medial hypothalamus (MH) because it is known to receive inputs from an SP pathway originating in the ME which, in turn, are relayed via a presumed, short brain projection to LH from MH. Significant dose and time dependent blockade of ME-induced suppression was observed. Phase III: microinjections of SP into MH (0.5, 1.0 and 2.0 mg/kg) resulted in significant (p < 0.05) suppression of LH-ejected QBA, thus mimicking the effects of ME stimulation. These results suggest that ME suppresses QBA by acting through an SP mechanism within MH.

NEUROPEPTIDES AND BEHAVIOR: OTHER


This study examines the effects of the non-NT receptor antagonist SR 48692 on some neurochemical and behavioral effects of NT agonists. In vitro studies, SR 48692, a non-NT receptor antagonist, inhibited the binding of [125I]NT to mouse preparations from 10-day old post-natal mouse brain and from HT 29 cells, but not from RINm5F cells. SR 48692 (300-3000 nM) blocked a dose-dependent increase in cyclic GMP levels evoked by NT (100 nM). In vivo, SR 48692 (0.5 and 5 pg/kg, i.p.) inhibited the increase in c-fos expression in the hippocampus (DOPAC/DOPA ratio) induced by the systemically active NT agonist, E-[N-MeArg-Lys-Pro-Tyr-seryl-Leu-Lys] doped at 2 mg/kg, i.p., 30 min after the SR 48692. No effects on dopamine turnover were observed in the striatum. In vivo, SR 48692 (1, 3 and 10 mg/kg, i.p.) did not antagonize the EI-induced (1 mg/kg, i.p.) decrease in spontaneous locomotor activity (LMA), the decreases in LMA induced by i.c.v. administered NT (0.05 to 0.1 mg/mouse), and did not inhibit the EI (0.1-1.2 mg/kg, s.c.)-induced fall in colonic temperature in mice. SR 48692 alone did not alter LMA or colonic temperature. These findings suggest the hypothesis that a subpopulation of the NT receptor mediates the locomotor and hypothalamic actions of this peptide and that it is different from the NT receptor that is involved in dopamine turnover.

OLFACTORY BULBECTOMY ALTERS LEVELS OF GALANIN AND NEUROPEPTIDE Y mRNA IN THE RAT LOCUS COERULEUS. P. V. Holmes*, U. Koprivica, and J. N. Crawford, Section on Behavioral Neuropharmacology, Experimental Therapeutics Branch, NIMH, Bethesda, MD 20892.

The noradrenergic locus coeruleus (LC) contains high levels of galanin (GAL) immunoreactivity and GAL mRNA and moderate levels of neuropeptide Y (NPY) immunoreactivity and NPY mRNA in the rat. LC neurons project to several forebrain areas, including the olfactory bulb. The effects of surgical ablation of the olfactory bulb (OBX) on levels of mRNA for GAL, NPY, and tyrosine hydroxylase (TH) in the LC and other brain regions were examined with quantitative in situ hybridization histochemistry. Increases in GAL mRNA levels in the LC were observed two weeks following bilateral OBX. Levels of NPY mRNA were slightly decreased and levels of TH mRNA were unchanged in the LC at this time point. Unilateral OBX increased GAL mRNA levels in the ipsilateral LC, suggesting that the elevation in GAL mRNA was a direct result of axotomy. Behavioral experiments confirmed previous reports of differences in open field activity between rats receiving bilateral OBX and sham operated controls. Furthermore, alterations in open field activity depended on the aseverness of the environment. The activity of rats with OBX was increased compared to controls under bright but not dim lighting conditions. Freezing behavior in response to footshock was attenuated in rats with OBX compared to controls. These results suggest that alterations in GAL and NPY mRNA in the LC and other brain regions may underlie the alterations in stress-related behaviors induced by OBX.
656.5 DISTRIBUTION OF NEUROPEPTIDE Y IN THE MALE SYRIAN HAMSTER BRAIN. D.B. Partin and S.W. Newman. Dept. of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48109-0616.

Pharmacologically distinct neuropeptide Y (NPY) in regulation of gonadotropin secretion and mating behavior. While the limbic pathways controlling mating behavior were defined in the male hamster, the pathway's neural substrates were not fully characterized. This study examined the distribution of NPY containing cells, fibers, and terminals in the male hamster brain. Fibers from colchicine (ICV injection, n=5) and non-colchicine male hamsters were cut into 30 mm coronal sections, and processed for immunocytochemistry using one of two primary antibodies for NPY (Incstar or donation from Dr. Marvin Brown, UCSD). The regional distribution and intensity of neuronal staining was the same in colchicine treated brains using either antibody. However, in non-colchicine treated brains, the intensity of neuronal staining was more robust with the Brown antibody than with the Incstar, when the distribution was unchanged. NPY cells were found in the deep layers of cortical structures throughout the telencephalon, including the olfactory bulbs, anterior olfactory nucleus, piriform cortex, and isocortex, as well as in region CA4 of the hippocampal formation. Cells were also distributed throughout the dorsal and ventral striatum. In the extended amygdala, fibers and terminals were observed in the central amygdaloid nucleus and the bed nucleus of the stria terminalis. Hypothalamic fiber plexuses and terminal fields were found in the medial preoptic area, paraventricular nucleus, suprachiasmatic nucleus and arcuate nucleus. Thus, NPY neurons and fibers distributed in limbic structures that relay chemosensory information to control mating may mediate this essential sensory processing. (Supported by NIH NS-20625, T-32-HD070408).


Galanin (GAL) and Neuropeptide Y (NPY) injected into the hypothalamus can induce feeding behavior. Moreover, feeding is known to cause changes in extracellular levels of dopamine (DA) and acetylcholine (ACh) in the nuclear accumbens (NACs) of rats. Therefore, the following experiments used microdialysis to study the effects of GAL and NPY injections into the paraventricular nucleus (PVN) on accumbens DA and ACh. Dialysis probes (2 mm) aimed at the NAC were inserted 24 hr before experimentation and DA or ACh was monitored. During dialysis each rat received counterbalanced injections into the PVN of GAL, NPY or saline. Following the dialysis procedure on separate days the effect of GAL, while NPY saline modified DA or ACh concentrations in the NAC even though NPY induced feeding in 6 of 12 rats. This result demonstrates that hyperfeeding of GAL activates the mesolimbic dopamine system and suggests that GAL could be involved in the reinforcement of feeding behavior. Supported by USPHS grant NS 30697.

656.7 DIPSOGIC DOSES OF ANGIOTENSIN II (AII) DO NOT AFFECT COGNITION, ACETYLCHOLINE (ACh) OR GABA RELEASE IN THE RAT. K. Szczepanski, A. Baxton, S.E. Daniels, P.A. McNeely, R.M. Johnson, and D.J. Pontius. Department of Neurosciences, Institute of Pharmacology, System Research, Palo Alto, CA 94303, U.S.A.

We assessed the behavioral and neurochemical action of renin and angiotensin II (AII) in the rat. We studied the behavioral effects of dipsogenic doses of AII and renin on learning and memory in two cognitive tasks: the Morris Water Maze and Passive Avoidance. All (0.1 and 1 μg, i.c.v.) did not affect latency to find the platform in the water maze. All (0.01 and 1 μg, i.c.v.) and renin (0.1 and 10 μg, i.c.v.) given 30 min before training, and AII (1 μg, i.c.v) given 5 min after training, did not affect retention in passive avoidance. These results suggest that while AII and renin are involved in thirst or fluid regulation, they do not affect learning or memory. In neurochemical experiments we used superfusion technology to study the action of AII on K+-evoked [H]ACh release and [H]-GABA release in slices of hippocampal and entorhinal cortex. Unlike Dop 996 (10 μM) which increased [H]-ACh release and [H]-GABA release in hippocampal and entorhinal cortex slices. AII did not affect release in hippocampal slices (1 μM) or entorhinal cortex slices (0.1 μM). These results suggest that AII does not affect ACh or GABA release. The findings from our behavioral and neurochemical studies suggest that either the brain renin-angiotensin system is not involved in cognition or it mediates cognition through a system other than the cholinergic or GABAergic system.

656.8 DISTRIBUTION OF ARGinine VASOTOCIN IN THE EARLY AMPHIBIAN EMBRYO. S.K. Boydst. Dept. of Biological Sciences, Univ. of Notre Dame, Notre Dame, IN 46556.

The neuropeptide arginine vasotocin (AVT) is widespread throughout the brain of adult amphibians. It is unknown, however, when AVT first appears during development. We used immunocytochemistry to map the distribution of AVT in early embryos of the South African Clawed Frog, Xenopus laevis. Staining was done in whole amphibious embryos or in cryostat sections of embryos, at ages from fertilization onward. AVT-immunoreactivity first appeared at the neural tube stage of development (Nieuwkoop and Faber stage 21). Cells (5-10) were located anteriad with strong fiber projections extending laterally. These cells were located at the rostral end of the neural tube, within the region which will form the brain. Scattered cells and fibers were also observed throughout the rest of the neural tube, within presumptive spinal cord stage 4-5, primary brain divisions are evident and the majority of AVT-ir was located in the diencephalon. Fibers were also observed in the developing eye. AVT-immunoreactivity thus appears very early in amphibian nervous system development. The homologous peptides in mammals, vasopressin and oxytocin, do not appear until considerably later in embryonic mammalian development. AVT in the early embryo may influence many aspects of neural development, including organization of sensory or motor circuits.

656.9 EXPRESSION OF AMPHIBIAN BRAIN THYROTROPIN-RELEASING HORMONE AND ARGinine VASOTOCIN RECEPTOR GENES IN Xenopus laevis OOCYTES. C.L. Mitchell, J.S. Holder, H.E. Eich and S.K. Boyd. Dept. of Biological Sciences, Univ. of Notre Dame, Notre Dame, IN 46556.

Thyrotropin-releasing hormone (TRH) and arginine vasotocin (AVT) are neuropeptides found throughout the amphibian brain. Although both have numerous mRNA expression receptors for TRH and AVT have not been fully characterized in any non-mammalian vertebrate. We expressed amphibian brain TRH and AVT receptors by injecting X. laevis oocytes with brain whole mRNA. Expression of receptors was measured by voltage clamping to monitor current flow across the membrane of oocytes in response to ligand. Injected oocytes were responsive to TRH, TRH free acid, cyclo-his-pro-[3-Meth]TRH and AVT, as indicated by inward membrane currents. The response was specific, since mRNA-injected cells showed no response to the non-peptide ligand, lanosin and un.injected or water-injected cells showed no change in current when exposed to any neuropeptides. The TRH response was reversible, dose-dependent, and saturable. Time course of response to maximally effective ligand doses was consistent. Latency (from ligand addition to onset of current flux) and response time (from onset of response to saturation) were characteristic of agonist application. Time course differences suggest that the amphibian brain expresses more than one subtype of TRH receptor. The % vasopressin receptor antagonist 4CH(Tyr(Me))AVP did not alter response when added to cells already maximally stimulated with AVT. When added alone, however, it produced a gradual current flux which was increased by secondary application of AVT. The mammalian antagonist thus appears to have agonistic effects in this system. We conclude that unique receptors for TRH and AVT and our data represent the first expression of these receptors from any non-mammalian vertebrate.

656.10 V1-RECEPTOR MEDIATED EFFECTS OF VASOTOCIN ON MOTIVATIONAL MNEmOnIC AND AVersIVE COMPONENTS OF SEXUAL LEARNING IN GUAL. S. Bernoindt* and St. Leudig, Institute for Zoology, University of Saalburg, A-6020 Saalburg, Austria.

Numerous studies have uncovered "homework constraints" imposed on adaptive specializations of sexual learning (Holloway & Domjan, J Exp Emeril Psychol, 1947-65, 1993). Behavioral mechanisms of vasopressin (AVP) could influence the motivational and aversive components of sexual learning. AVP effects on sexual behavior of females during five successive days, showed an inhibitory action of AVT to the reinforcing effect of copulatory "award" following visual access. Again this effect was dose dependent.

A dissociation of peptide treatment from copulation experience for up to 6 hours did not change AVT effects on prosex behavior during the following day: together with a composition of training effects a positive reinforcement of social proximity by copulation, the experiments provide evidence for AVT action on motivational mechanisms of sexual learning. These effects appear to be mediated by receptors homologue to mammalian vasopressor AVP-V1.
Chlorodiazepoxide, Diazepam (mg/kg), which was administered at 12:00, was known to induce behavioral changes. We previously reported that IL-1β, IL-2 and IL-6 induced cytokine-specific alterations of central neurotransmitter activity. We presently report that cytokine-induced behavior was also evident in male BALB/c mice following peripheral administration of these cytokines. Exploration of a novel environment (i.e., locomotion, rearing, digging) was significantly decreased by 15-50 minutes after IL-18 administration (200 ng, ip). In contrast, IL-2 and IL-6 induced behavioral activating effects. IL-2 (50, 100, 200 or 400 ng ip) induced dose-dependent increases in time spent in non-ambulatory exploration, number of rears and distance travelled in a novel environment. IL-6 dose-dependently (50, 100, 200 or 400 ng ip) increased the number of rears, digging episodes and the time spent per episode as well as the number of grooming episodes. Moreover, time-dependent increases in the number of contacts with a novel stimulus and mean-contact time were evident in mice administered IL-2, but not in IL-6-treated animals. Taken together, these data suggest that IL-1, IL-2 and IL-6 induce cytokine-specific behavioral alterations. The results and discussed in terms of the spectrum of behavioral alterations induced by immune-derived products and implications on the characterization of sickness behavior. (Supported by NIMH and MRC of Canada)

**NITRIC OXIDE MODULATES RETENTION IN THE PORSOLT SWIMMING TEST.** D. Jeffreys* and J.W. Funder. Baker Medical Research Institute, Melbourne, Victoria, Australia 3181

In the Porsolt swim test intact animals become progressively immobile over a 15 min test period and 248 hours later are immobile for ~70% of the 5 min rest period. Adrenocorticotropin (ACTH) blocks retention of the response, with animals remaining immobile for only ~35% of the rest period, an effect reversed by Dexamethasone (6 μg) or Ketocyclazocine (1 mg/kg). When the antagonistic RU38466 is administered to intact rats it is without effect, as is MR2266, a kappa opioid antagonist. In contrast, when RU38466 and MR2266 are simultaneously administered the animals have similar levels of immobility on retest as in ACTH. Retention of the immobility response thus can be directly mediated by glaucocorticoid or kappa opioid pathways.

In the present studies we show that N-nitro-L-arginine (NAME), a nitric oxide inhibitor dose-dependently blocks retention, with animals remaining immobile for 35-42% on retest at a dose of 50 mg/kg. This effect of NAME is blocked by L-arginine (50 mg/kg), with animals immobile for 77% on retest. NAME is effective given immediately pre-test, but was without effect administered 15 min post-test. When ACTH was given L-arginine the effect of adrenocorticotropin was blocked, with animals remaining immobile for 62% on retest. These findings suggest that both glucocorticoid and kappa opioidergic effects on retention are mediated by a final common pathway involving nitric oxide.
565.17


The effect of the central administration of nitroglycerin, a potent organic nitrate vasodilator, on penile erection and yawning was studied in male rats. Following low doses of nitroglycerin (33 - 99 µg) induced penile erection and yawning in a dose-dependent manner. Nitroglycerin-injected penile erection and yawning effects were also injected in the paraventricular nucleus of the hypothalamus, a brain area that plays a key role in the control of these responses induced by agents such as N-methyl-D-aspartate, dopamine agonists and oxytocin. Nitroglycerin-induced penile erection and yawning were prevented by methylene blue (400 µg), d(CH2)5Tyr(Me)2-Orn9-vasotocin (0.1 µg) but not methemoglobin (100 µg) given ICV. In contrast these compounds were ineffective when injected in the paraventricular nucleus. Ineffective was also haloperidol (1 mg/kg IP). The results suggest that nitroglycerin induces penile erection and yawning by activating oxytocinergic transmission through the production of NO in the paraventricular nucleus of the hypothalamus.

565.19

EFFECTS OF PRENATAL STRESS ON DEFENSIVE WITHDRAWAL IN THE RAT. HE Ward, EA Johnson, DL Birkle, MS Cratty, AJ Azzaro*. Dept.s of Behavioral Medicine/Psychiatry, Pharmacology/Toxicology, and Neurology, West Virginia University School of Medicine, Morgantown, WV 26506.

Adult, virgin, female Sprague-Dawley rats were bred with adult males. Between gestational day 14 and 21, pregnant females were exposed to the mild stress of daily handling and saline injection (0.1 ml, s.c.). Control (unstressed) dams were not handled except for normal animal care. Behavioral testing in a defensive withdrawal apparatus was performed on male offspring at 60 days of age. There were no significant behavioral differences noted between control and prenatally stressed animals. However, following 2 hr of restraint stress, prenatally stressed offspring had a longer latency to exit from the defensive withdrawal chamber and spent less time in the open field (n=8, p<0.05). Specific binding of corticotropin-releasing factor (CRF) was increased by 30% in the amygdala of the prenatally stressed animals (n=8, p<0.05). CRF levels and secretion were similarly elevated (Cratty et al, this volume). In the defensive withdrawal paradigm, without an acute stress (restraint), behavioral differences were too subtle to detect. However, it would appear that the animals ability to adapt to acute stress (restraint) was compromised by prenatal stress and may be a function of prenatal perturbation of development CRF systems within the amygdala. Supported in part by NIH (HD079R50343-31 and MH19444) and UHA.

565.2

AN ENDOGENOUS INCREASE IN CYCLIC GMP IS ASSOCIATED WITH INCREASED EXCITABILITY IN IDENTIFIED NEUROSECRETORY CELLS IN MANDUCA SECTA. Stephen C. Gamme, John Everall, and James W. Truman*. Department of Zoology, University of Washington, Seattle WA 98195.

Ecdysis in insects is triggered by the neuropeptide, ecdysis hormone (EH). During the course of EH action in the tobacco hornworm, Manduca sexta, a group of neurosecretory cells show a dramatic increase in guanosine 3'5' cyclic monophosphate (cGMP). This increase precedes the onset of the ecdysis behavior and is sustained at high levels in some of the neurons for over 2 hours. Using intracellular recording techniques we have found that this endogenous increase in cGMP is correlated with a significant lowering of the threshold of these neurons. Also, the somata of these cells show a calcium dominated action potential and this is lengthened during the time of cGMP increase. We found, however, no changes in either input resistance, resting potential, or action potential amplitude to be associated with cGMP expression.

Currently, we are injecting cGMP into these neurons prior to their endogenous increase in cGMP to directly test the actions of this cyclic nucleotide on cell excitability. These experiments will be followed by whole cell voltage clamp techniques to determine which ionic currents are affected and how.

565.18

COMPARISON OF THE RESPONSE OF 4 HIPPOCAMPAL (H) SITES IN DIAZEPAM (DZ) DEPENDENT RATS TO FLUMAZENIL (F) MICROINJECTIONS. J.W. Sloan*, E.P. Wake and X. Jing, Dept. of Anesthesiology, College of Medicine, Univ. of Ky., Lexington, Ky., 40536.

In order to identify sites in the female Sprague-Dawley central nervous system that contribute to the development of DZ dependence, guida cannulae (GC), also serving as recording electrodes, were implanted into the occipital cortex (OCT, area 2), mediodlimal (MD) (AP=3.8; RL=2.4; V=8) or CTX (area 2, lateral [LAT]) (AP=3.8; RL=4.5; V=8). Electrodes were implanted into the H (AP=3.7; RL=2; V=6) and frontal CTX (AP=10.7; RL=1; V=10). After recovery, the rats were microinjected subcutaneously with 30 µl saline solution filled with 180 µg DZ/cap. After the 3rd implant and the attainment of stable plasma levels of DZ and its metabolites, rats were microinjected weekly with F (25 µg in 1 µl of DMSO) or DMSO into the H via GC. EEGs, behavioral (BEH) and precipitated abstinence scores (PAS) were recorded prior to and for 40 min after microinjection. When F was microinjected via GC into area 2, MM of OCTX with chemotrode (CTR) in CA1 of H, V=7.2, n=5 rats) neither BEH nor PAS were elevated whereas convulsive signs (switches and jerks), head and body tremors and respiratory rate were increased. When the CTR was lowered into dentate gyrus, V=6.2 (n=4), F produced no significant signs of EEG. The case when F was microinjected via GC in area 2, LAT of OCTX through CTR into H, V=5.8 (n=5), although 1 rat had a clonic convulsion. In contrast, when F was microinjected via same GC through CTR in CA1 of H, V=6.8 (n=8), BEH and PAS were elevated. Convulsive activity was apparent both by observation and EEG in some but not all rats. These data indicate that DZ given chronically produces dependence in some but not all areas of the H and that the CA1 area of the dorsal H is involved. Supported by NIDA Grant DA02195.
HISTOLOGICAL AND ULTRASTRUCTURAL BRAIN ALTERATIONS OF A MAN CRONICALLY EXPOSED TO HIGH DOSES OF DIAZEPAM. M.C. Márquez-Orceño,* F. R. Pérez-Chuqui, M. V. Gasca-Herrera, and A. Márquez-Orceño. Embryology Dept., Medicine School BUAP, Mexico 04510 D.F. and BSZ FPT 2-4, INSS.

Our purpose was to investigate whether the histological and histoelectronultrastructural brain alterations observed in a 50-year-old man, exposed to daily diazepam (20 mg/kg) during the last ten years of her life, are similar to those observed in adult mice prenatally treated with DZ. Cerebral and cerebellar cortices and striatum samples were studied with a light and transmission microscopes. The causes of death were not clearly determined. Pathology autopsy showed generalized cortical atrophy, without pre-semi or semi dementia and premature aging. Histological and ultrastructural sections showed II y III cortical layers disorganized presence of abundant sylodeysis bodies, perinuclear vacuolization. Less number of Purkinje cells than in cerebellum of three healthy men from the same age. In all neural cells the heterochromatin was seen in dense clumps scattered in its interior around the nucleus and mainly located near the nuclear envelope. The euchromatin, was reduced. Golgi complex, mitochondria, rough endoplasmatic reticulum and synaptic vesicles were disorganized. Electrondense bodies were observed. These alterations are similar those showed in adult mice prenatally exposed to DZ and suggest may be due to chronic exposure to high doses of DZ.


This experiment is part of a study of the effects of prenatal exposure to the benzodiazepine [Diazepam (DZ), Lorazepam (LZ)] on the behavior, EEG and brain-specific receptors of the mature offspring. Twenty adult female Sprague-Dawley rats received 1 mg/LZ/kg S.C. or the equivalent volume of vehicle, once daily, for 28 days prior to breeding, during breeding and throughout their 21-day gestation. The behavior of the offspring of both sexes was evaluated at 34 months of age. We used a modified radial arm maze test which incorporates measures from the open field test, hypnoencephalographic model of anxiety test, Olton's original RAM test and other parameters of our own design. Prenatal exposure to lorazepam produced performance deficits indicating a long-lasting state of hyperarousal or "anxiety" in the male offspring. Conversely, the female offspring prenatally exposed to lorazepam show enhanced performance that may indicate a reduction of "anxiety" relative to controls. Thus, prenatal lorazepam exposure has produced an exaggeration of the normal sex-dependent differences in maze performance and spontaneous behaviors. These results could result from an elevation of sex steroid levels or an alteration of the interaction of sex steroids with the benzodiazepine receptor which enhances the efficiency of circulating steroids.

BENZODIAZEPINE LIGANDS MODULATE ETHANOL DRINKING IN ALCOHOL-PREFERING AA RATS. E.R. Kopp* K. Weegels, T. Oyasaki and A. Honkonen. Biomedical Research Center, Alko Ltd, POB 350, FIN-00101 Helsinki, and Department of Pharmacy, Division of Pharmacology and Toxicology, University of Helsinki, Finland.

The effects of positive allosteric modulators (agonists) at the benzodiazepine site of the GABA receptor (midazolam, abecarnil, KZ 92926, bretazenil, and CDS 9895) and those of negative allosteric modulators (antagonists, Ro 15-4513 and Ro 19-4403) were studied on voluntary ethanol consumption in selectively-bred alcohol-prefering AA rat line using limited access paradigm. Each drug was tested after IP injections of three different doses using saline injections as a control treatment. The benzodiazepine agonists had only modest effects in reducing intake, measured 2, 1 and 4 h after the injections, whereas the inverse agonists strongly decreased ethanol consumption. Food consumption during the 4-h session was decreased by the highest dose of the benzodiazepine agonist, which also significantly increased the ethanol drinking. The inverse agonists had no significant effect on food intake. Pentoxicon-sensitive ["STIBPS binding was differentially modulated by benzodiazepine ligands between the AA rats and alcohol-avoiding strains. [HJIU154513] binds to both sites but not, however, reveal any drastic differences between the lines. In conclusion, the results suggest that the GABA receptor may be involved in the regulation of ethanol drinking in酒精-prefering AA rats, but do not indicate that these rat would consume ethanol because of its axiolytic effects in a way that benzodiazepine agonists (full or partial) could substitute for it.


We investigate whether diazepam (DZ) caused ultrastructural alterations in fetal mice cerebral cortex. Three CD-1 strain gestating pregnant were observed in the 6th to 17th gestation day. One group with single daily DZ doses (2.7 mg/kg), the second with 0.7% saline solution (S) and the third was not treated (NT). All were killed with CO2 atmosphere the 18th day and the fetus removed. The cerebral cortex was fixed and embedded. Fine sections were observed under transmission microscope. In the DZ fetal mice cerebral cortex was evident delay cellular and neuropile differentiation. The nuclear density per millimeter was thinner in the S and NT fetuses (p <0.05). Heterochromatin was seen in clumps scattered mainly in its interior and near of the nuclear envelope. The rough endoplasmatic reticulum showed distended cisternae, the Golgi complex, the mitochondria and the polyribosomes were more abundant. Such alterations could reveal disruption in cell multiplication and so it could be related to diazepam-induced protein synthesis and to the modification of the metabolic pathways mediated by central and peripheral types of benzodiazepines receptors. The results suggest that DZ produce long-lasting ultrastructural alterations in the cerebral cortex.

LONG-LASTING EFFECTS OF PRENATAL EXPOSURE TO DIAZEPAM ON SEXUAL BEHAVIOR OF MALE MICE. L.A.I. Hernández-Alvarez,* A. Martinez-Vargas, B. Victoria-Romero, A. Márquez-Orceño and M.C. Márquez-Orceño. Dept. of Embryology, School of Medicine, BUAP and Dept. of Embryology, School of Medicine, UNAM. Mexico City, Mexico.

Effects of prenatal exposure to diazepam (DZ) in adult mice were showed on sexual reproductive behaviors. We assessed the effects of diazepam (DZ) on the behavior of male mice from the senile rats of the CD-1 strain mice exposed to DZ during gestation. One group of female mice was exposed to periodic doses of DZ (2.7 mg/kg/bw) from the 6th to 17th gestation day and control group received saline solution. On the 27th month of age, the spontaneous male sexual activity to females from the treated and control rats was tested and videorecorded under red light. Precopulating and copulating activities were evaluated. No difference was found in precopulating behaviors from both groups. During copulating stage, greater sets of mount series with and without penile intromission, as well as fail and interruptions of intravaginal penetration were found in DZ animals. Although ejaculations per animal were unusually in both groups, DZ mice had fewer. Results show a long-lasting effect of prenatal exposure to DZ of sexual behavior.
657.9
COMPARISONS OF [3H]Ro15-4513 BINDING IN CEREBELLUM FROM ETHANOL-NAIVE WITHDRAWAL SEIZURE PRONE AND WITHDRAWAL SEIZURE RESISTANT MICE. A. Leslie Morris*, F. Donelson Smith, and Leslie L. Devay, UNC Sch. of Medicine, Chapel Hill, NC 27599.

Ethanol withdrawn seizure resistant (WSR) and withdrawal prone (WSP) lines of mice have been selectively bred for investigation of the mechanisms involved in alcohol withdrawal. Ethanol-naive WSR mice are more sensitive than WSP mice to GABAergic chemosensitivants, suggesting differences in GABA_A receptor function or structure are involved. WSR mice exhibit 50-100% higher levels of GABA_A receptor subunits α1, α6 and β2 subunit mRNAs in cerebellum, but not cortex. Ro15-4513 is a GABA_A receptor inverse agonist which labels recognition sites on α6 subunits. The present study was conducted to determine if there are differences in [3H]Ro15-4513 binding characteristics between WSR and WSP mice. Saturation binding analysis was conducted with [3H]Ro15-4513 (0.2-20 nM) using individual cerebellum from WSR1, WSR2, WSP1 and WSP2 mice. No differences in binding were observed between the WSR1/2 or the WSP1/2 lines, respectively (n=8). Moreover, no differences in [3H]Ro15-4513 binding were observed in cerebellum from ethanol-naive WSR vs WSP mice. Binding density was 1467 ± 52 fmol/mg protein in the WSR and 1543 ± 41 fmol/mg protein in WSP cerebella. Kd values were 2.90 ± 0.09 nM and 2.82 ± 0.11 nM for WSR vs. WSP mice, respectively. These data indicate a discrepancy between the expression of GABA_A receptor α6 subunit mRNAs and [3H]Ro15-4513 recognition sites in these mice. (Supported by AA09913, ES07126 and Pharm. Man. Assoc. Found.)

657.11
THE BENZODIAZEPINE ANTAGONIST CGS-8216 EXERTS PROLONGED AND SELECTIVE ATTENUATION OF ETHANOL INTAKE IN ALCOHOL-PREFERRING (P) RATS. J. Williams, H.L. Jane, S. Dejevars, M.J. Lewis*, and J.M. Neisewander, Inst. of Psychiatric, Res. and Program in Medical Neurobiology, Indiana University-Purdue University, Indianapolis, IN 46202 and Dept. of Psychology, Temple Univ., Phila., PA 19122.

The present study investigated dose dependence and time course profiles of CGS-8216 (0.0, 0.5, 1.0, 5.0, 10, or 20 mg/kg), a pyrazoloquinolone BDZ antagonist in attenuating EtOH intake in alcohol-prefering P rats (N = 13). Animals were provided a 4 hr daily limited access to a two bottle choice between EtOH (10% v/v) and saccharin (0.0125% g/v) solutions. For the remaining 20 hr, rats were provided unlimited access to water, with food available ad libitum. Acute administration of CGS 8216 (1.0-20 mg/kg) dose-dependently reduced EtOH intake to 42± 7% of controls at the initial 15 min interval of the 4 hr access on Day 1. All doses reduced total consumption for the 4 hr period to 15-60% of control levels. On Day 2 (24 hr post-drug administration), the 5.0 and 20 mg/kg doses of CGS 8216 reduced EtOH intake to 41% of controls during the initial 15 min interval, while total consumption was reduced to 20-30% of control levels for all doses except the 10 mg/kg dose. Saccharin drinking generally showed no changes at the initial 15 min interval, however, compensatory increases paralleled the decreased EtOH consumption and the total consumption measure for several of the CGS 8216 doses. These results suggest the BDZ component of the GABA_A receptor complex may play a direct role in the reinforcing actions of EtOH. (Supported in part by grants AA08553 and AA07611.)

657.13
THE EFFECT OF ANXIOLYTICS ON NOVELTY-INDUCED PLACE PREFERENCE. J.E. Klabaure, D. Miller, M.T. Bardo, Department of Psychology, Univ. of KY, Lexington, KY 40506-0044.

Previous evidence has shown that rats exposed to one compartment of a place preference apparatus will prefer the novel compartment over the familiar. While this suggests that novelty is rewarding, an alternative interpretation is that rats avoid the familiar compartment because it is associated with stress induced during the inescapable exposure sessions. To test this latter possibility, the anxiolytics diazepam (0.1, 0.3, 1.0 mg/kg) and gepirone (0.1, 0.3, 1.0 mg/kg) were examined for their ability to alter novelty-induced place preference in rats. As expected, control animals showed a novelty-induced place preference. On test day, this preference was blocked by diazepam, but only at a dose (1.0 mg/kg) that also decreased locomotor activity. Gepirone failed to alter the preference behavior, even at a dose (1 mg/kg) that decreased locomotor behavior.

These experiments indicate that preference for the novel compartment is an indication of the rewarding effects of novelty and not an avoidance of stress associated with the familiar.

657.10

The present study investigated the extent to which possible genetic differences, which are phenotypically expressed as differences in EtOH preference, may influence sensitivity to the BDZ partial inverse agonist, RO19-4603 (Ro) in modifying low to high doses of EtOH (0.125-2.5g/kg). P (N=20) and NP (N=20) rats of the P1 strain were habituated to a photocell apparatus and tested for 10 min sessions during the experimental phase. Animals from each line were randomly assigned to an EtOH only group, or to a Ro+ EtOH group (n=10/group). Animals in both groups received three daily consecutive EtOH injections. Animals in the R group, however, were given R (0.15 mg/kg), 5 min prior to the EtOH injection on Day 1 only. EtOH dose caused dose-dependent reduction in motor behaviors on Day 1-3 (50 to 70% of control), with tolerance seen only with the NR rat for the 0.125 g/kg dose on Day 2. Ro enhanced the suppressant effects of the 0.125 g/kg EtOH dose in the P and NP line rats on Day 1. R was without effect on Day 1-3 when given before the 1.0 and 1.5 g/kg EtOH dose in the P rats, as well as Day 1 in the NP rats. R antagonized, however, the EtOH suppression on Day 2 to 3 in the NP rats with the 1.5 g/kg dose, as well as Day 1 and 3 with the 1.5 g/kg dose. It was without effect on the 1.5 g/kg dose of EtOH in the P or NP rats or given alone. The results suggest R may induce rapid changes at the GABA_A receptor and mediate the motor-imparing effects of EtOH for 48 hr in the P2 line NP rat, but not P rat. These effects may be related to the low EtOH preference of the NP rat. (Supported in part by grants AA08553 and AA07611.)
DRUGS OF ABUSE: ALCOHOL AND OPIOIDS

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658.1
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Brain opioid systems may have a role in the ethanol reward. Our aim was to study possible differences in the sensitivity of these opioid mechanisms of alcohol-prefering (AA) and alcohol-avoiding (ANA) rats by activating the central opioid mechanisms with ethanol, morphine, and morphine-stimulating stress followed by measurement of the analgesia induced by these treatments. Analgesia was measured with the tail-flick (TF) test. The baseline TF-latency was measured 4 times with 5 min intervals before treatments. The mean of the last 3 readings was used as baseline (BL). Ethanol (15 %, i.p., 1.0 or 2.0 g/kg) or saline was given after the last BL trial. TF-latency was measured 15, 30, and 60 min after injection. The TF-latency was measured just before each injection. Stress was induced to rats by exposing them to 3 min swimming in 15°C water. TF-latencies were measured just before and 3, 6, and 9 min after swimming. TF-latencies increased in the AA rats during 4 BL-trials but not in the ANA rats. No difference was found in the MO or stress induced analgesia between rat lines. The smallest ethanol dose (1.0 g/kg) failed to produce analgesia in either rat line, but 1.5 g/kg of ethanol produced slight analgesia in the AA rats but not in the ANA rats. Both rat lines showed similar analgesia after 2.0 g/kg ethanol. These results suggest that the ANA rats may be more resistant to habituate to repeated nociceptive stimuli than the AA rats. The AA rats appear to be slightly more sensitive to analgesic effect of ethanol than the ANA rats. Whether this effect is due to greater release of endogenous opioids in these rats remains to be studied.

658.2
Dept. Molec. Pharmacol. & Toxicol., School of Pharmacy, Univ. of Southern California, Los Angles, CA 90033

Exposure to 12 atmospheres absolute (ATA) helium-oxygen gas mixtures (heliox) antagonizes a broad range of brain stimulatory and depressant effects of ethanol. If pressure antagonizes ethanol by blocking or offsetting its initial molecular actions, then the antagonistic effects of pressure should be limited to drugs acting via similar mechanisms. The present study tested this hypothesis by extending preliminary investigations of the effects of hyperbaric exposure versus morphine-induced locomotor activation. Drug-naive C57BL/6J mice were habituated to BL and measured ethanol (10-32 mg/kg) and then exposed to 12 ATA helium. Morphine injection (1 mg/kg) was given i.p., 15 min prior to the initial measurement of hypothermia. Morphine dose-dependent stimulation of locomotor activity in response to ethanol was observed. The number of 10 sec loco-events (i.e., rears) was increased in response to ethanol with or without morphine. The data indicate that the effects of hyperbaric exposure on the ethanol-induced locomotor activity may be attributable to a specific antinociceptive effect of pressure on the ethanol-related behavioral activity. These results suggest that the use of hyperbaric exposure may provide a novel method for the treatment of ethanol-related disorders.

658.3
Molecular Pharmacology and Toxicology, School of Pharmacy, University of Southern California, Los Angles, CA 90033

Low-level hyperbaric exposure antagonizes several ethanol-induced behaviors including ethanol-induced motor depression and anticonvulsant effects. Recent work has also found that pressure also antagonizes diazepam-induced locomotor depression suggesting that ethanol and diazepam may have a common pressure antagonizable site of action. The present study further explored this hypothesis by testing whether pressure was related in the pressure effects and treatment to alanova injection of 300 mg/kg of INH. The mice were then exposed to either 1 atmosphere absolute (1 ATA) air or 1 ATA helium-oxygen gas mixture (heliox) or 12 ATA helium at temperatures that offset the hypothermic effects of helium. Diazepam increased the latency to onset of myoclonus in a dose dependent manner. Exposure to 12 ATA helium antagonized diazepam's anticonvulsant effect at 8.0 and 24.0 mg/kg, but not at 4.0 mg/kg. Diazepam also increased the latency to onset of clonus in a dose dependent manner. Diazepam antagonized this anticonvulsant effect. These findings extend the acute behavioral effects of diazepam known to be antagonized by hyperbaric exposure and support the hypothesis that ethanol and diazepam share a common pressure antagonizable site of action in the GABA_A receptor complex (Supported by NIAAA grant AA09792).

658.4
Alcohol Preference in Rats Selectively Bred for Saccharin Consumption. N. E. Badia-Elder*, S. W. Kiefer, and N. K. Davis.
Psychology Dept., Kansas State Univ., Manhattan, KS 66506, Psychology Dept., Occidental College, Los Angeles, CA 90041

Selectively bred high saccharin consuming (HIS) and low saccharin consuming (LOS) rats, obtained from The Occidental College in Los Angeles, were tested for taste reactivity to 10% alcohol, sucrose, quinine, and a sucrose/quinine mixture. After taste reactivity testing, a two-bottle consumption test with 10% alcohol and distilled water was given for 14 days. At the end of two-bottle testing, rats were tested a second time for taste reactivity to 10% alcohol. In the initial taste reactivity exposure, HIS and LOS rats did not differ in response to 10% alcohol, sucrose, quinine, or the sucrose/quinine mixture. Likewise, there were no significant differences between HIS and LOS rats in their second taste reactivity to 10% alcohol. However, there was an exposure effect in that all rats significantly increased ingestive responding and decreased aversive responding to 10% alcohol on the second test. HIS and LOS rats did not differ in alcohol consumption as measured with two-bottle testing. According to these results, HIS and LOS rats display similar patterns of alcohol preference as measured by taste reactivity and consumption (Supported by Training Grant T 32 MH 19547 from NIMH to the Society for Neuroscience).

658.5
Dept. of Psychology, Kansas State Univ., Manhattan, KS 66506-3302

Previous work reported that Low Alcohol Sensitive (LAS) rats failed to develop normal saccharin aversions, relative to High Alcohol Sensitive (HAS) rats, when alcohol was used as the illness agent. In the present experiment, HAS and LAS rats were compared with control rats in the acquisition of an alcohol aversion using lithium chloride as the illness agent. Rats were trained to avoid a 6% alcohol solution by pairing it with LiCl intubations (3% body weight of 0.15 M solution). Rats were tested for taste reactivity to 5% alcohol on the day after training. This test was then followed by extinction trials with no alcohol in the drinking situation. No significant differences between groups were found in ingestive or aversive taste reactivity. There were also no significant consumption differences found between groups during these data. The data indicated that HAS and LAS rat lines are capable of developing normal alcohol aversions when the illness agent is LiCl. (Supported by Training Grant T 32 MH 19547 from NIMH to the Society for Neuroscience).

658.6
Naltrexone and Taste Reactivity to Alcohol In Rats. K.G. Hill*, N.E. Badia-Elder, and S.W. Kiefer.
Dept. of Psychology, Kansas State Univ., Manhattan, KS 66506-5302

Naltrexone, an opiate antagonist, has been shown to reduce alcohol consumption, particularly in high alcohol consuming rats. In the present study, we determined whether naltrexone altered the taste reactivity responding of rats when presented with an intragastric infusion of alcohol. Two groups of rats were tested for their taste reactivity response to 10% alcohol 30 min after naltrexone treatment (1 mg/kg) and 10 min after saline treatment: High Ingestive Responding (HIR, n=7) rats, which were selectively bred for their taste reactivity to alcohol and which consumed high amounts of 10% alcohol; Sprague-Dawley (n=8) rats that had previous experience with a range of alcohol concentrations. Although naltrexone did not alter alcohol reactivity in the Sprague-Dawley rats, HIR rats did exhibit increased aversive responding and decreased ingestive responding following naltrexone treatment. The results provide preliminary evidence that naltrexone may alter alcohol consumption by altering its palatability. (Supported by Training Grant T 32 MH 19547 from NIMH to the Society for Neuroscience).
NALTREXONE ATTENUATES ETHANOL STIMULATED DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS. R.B. KOHL and W.J. McBride*. Depts. of Psychiatry and Medicine, Medical Neurobiology Graduate Program, Indiana University School of Medicine, Indianapolis, IN. 46223-4887.

The effect of naltrexone (NAL) on ethanol stimulated-dopamine (DA) release in the nucleus accumbens was studied in adult, female, Wistar rats using in vivo microdialysis in conjunction with HPLC with electrochemical detection. Rats were implanted with microdialysis probes 36-48 hours prior to the administration of ethanol alone (2.0 g/kg, i.p.) or ethanol following pretreatment with 20 mg/kg i.p. NAL. Experiments were conducted on awake, freely moving animals. The i.p. administration of 2.0 g/kg ethanol increased extracellular DA concentrations to approximately 160% of baseline (N=6) within 60 minutes and levels remained elevated for at least two hours. The i.p. administration of NAL (N=5) 30 minutes before injection of ethanol reduced (p<0.001) the ethanol induced increase in extracellular DA concentrations. There were no effects on extracellular levels of dopamine with i.p. injections of saline. In general, the extracellular levels of DA and serotonin metabolites did not change during any of the treatments. The results suggest a role for endogenous opioids in mediating the actions of alcohol on the mesolimbic DA system. (AA 00900, AA 08533)

NALOXONE FAILS TO BLOCK INHIBITORY EFFECTS OF ETHANOL ON SPONTANEOUS AND AMYGDALA-INDUCED SINGLE UNIT ACTIVITY OF NUCLEUS ACCUMBENS NEURONS. R.S. Lee, J.S. Criado, C.I. Berg, and S.L. Merchenthaler, Alcohol Research Center, Dept. of Neuropharmacology, Scripps Research Institute, La Jolla, CA 92037.

Previous studies from our group have shown diverse effects of ethanol on nucleus accumbens (NAcc) physiology. For example, systemic administration of ethanol reduces the firing rate of spontaneous and glutamate-activated NAcc neurons in both acute anesthetized and unrestrained rats. It has not been determined whether the effect of alcohol on the firing rate of these NAcc neurons is mediated by the reinforcing properties of ethanol. In the current study we examined the inhibitory effects of alcohol on ethanol-dependent injection of glutamate-activated NAcc neurons. In our experiments the firing rate of NAcc neurons was significantly reduced by ethanol (1.2-1.4 g/kg, i.p.) and has been significantly reduced by ethanol (1.2-1.4 g/kg, i.p.) and has been significantly reduced by ethanol (1.2-1.4 g/kg, i.p.) significantly reduced the firing rate of spontaneous and glutamate-activated NAcc neurons in both electrophysiological preparations. Similarly, ethanol inhibited the occurrence of amygdala-activated single unit in NAcc neurons. Naloxone did not reverse these inhibitory effects. These data indicate that the inhibitory effects of ethanol on NAcc physiology are independent of opioid mechanisms (supported by ARC AA06420 to SJH).

THE EFFECT OF NALTIRINDOLE ON EXPRESSION OF ETHANOL-INDUCED CONDITIONED PLACE PREFERENCE. S.D. Dickinson and C.L. Cunningham*. Dept. of Medical Psychology, Oregon Health Sciences Univ., Portland, OR 97201.

Recent research has suggested a role of the endogenous opioid system in the reinforcing properties of ethanol. Research in our laboratory has shown that the expression of ethanol-induced conditioned place preference in mice can be attenuated by naltirindole (1.5 - 10 mg/kg). However, at these doses, naloxone may act as a nonspecific antagonist, occupying both mu and delta opioid receptors. The current study was designed to determine whether naloxone's actions were delta-receptor mediated. Three groups of male DBA/2J mice underwent a Pavlovian conditioning procedure that paired injection of ethanol (2 g/kg, IP) with a distinctive tactile stimulus and injection of saline with a different stimulus. After four pairings of each type, a preference test was conducted in which mice were exposed to both tactile stimuli. Prior to the preference test, mice were injected with either 0, 3, or 10 mg/kg of naltirindole (NTI), a selective delta opioid receptor antagonist. All groups demonstrated a conditioned preference for the tactile stimulus that had been paired with ethanol. Unlike naloxone, NTI had no effect on the expression of the conditioned preference. This indicates that naloxone's ability to interfere with expression of ethanol place preference is not mediated via delta opioid receptors. Supported by an N. L. Tartar Fellowship.

IBOGAINE ATTENUATES ALCOHOL INTAKE IN ALCOHOL DRINKING RATS. Y.W. Lee, Amir H. Rezaei* and D.H. Overtstreet. Skinner Bowles Center for Alcohol Studies, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7175.

Bogaine, the principal alkaloid of the root bark of Tabo subermuthi grown in West Central Africa, has been claimed to possess anti-addictive properties for cocaine and morphine. This study was designed to determine the effect of Ibogaine on alcohol intake in two strains of alcohol drinking rats. Alcohol preferring (P) and Fawn Hooded (FH) rats were injected IP with either vehicle or one of the doses (10, 30 and 60 mg/kg) of Bogaine and their alcohol intake was measured 24 hr later. In the experiments the effects of acute and subchronic intragastric as well as acute subchronic administrations of Bogaine in FH rats were determined. Intraperitoneal injections of Ibogaine significantly and dose-dependently attenuated alcohol intake in both strains. However, FH rats were less affected by the drug. Subchronic administration of the drug did not exert any effect. Both acute and subchronic intragastric administration of 60 mg/kg Bogaine significantly reduced alcohol intake without development of tolerance. The fact that Bogaine reduced alcohol intake when injected intraperitoneally and intragastrically, but not subchronically, suggests the involvement of its metabolite(s) in reducing alcohol intake. Although the true mechanism(s) of action of Bogaine in reducing voluntarily alcohol intake is not yet clear, the fact that FH rats with genetic serotonin dysfunction were less affected by this drug suggests that, in addition to other neurotransmitters, Bogaine may interact with serotonergic systems in the brain to reduce alcohol intake.

EFFECT OF CHRONIC ALCOHOL ADMINISTRATION ON DIURNAL ACTH AND CORTICOSTERONE SECRETION IN INTACT AND ADRENALECTOMIZED RATS WITH CORTICOSTERONE REPLACEMENT. T. Chevret, T. Torres, R. Deval, J.G. Ordone*, and R. Estany, NIAAA, NIH, Bethesda, MD 20892.

The hypotalamo-pituitary-adrenal (HPA) axis is characterized by a circadian variation of corticosterone (CS) and ACTH levels near lights on and highest hormone levels near lights off. Intact or adrenalectomized (ADX) male rats Implanted with CS pellets (plasma CS levels of 3 to 6 µg/dl) were fed every four hours for up to 10 days via an intragastric cannula with the Leiber-DeCarli liquid food diet (LCD) or ethanol diet (LED). Blood samples were taken via a carotid catheter at the trough of the circadian rhythm. In intact alcohol-treated rats, daily plasma CS levels were 2 to 10 fold higher at the nadir, when compared to control rats, whereas daily plasma ACTH levels at the nadir were elevated less than 50% of the time. In ADX rats with CS replacement, plasma ACTH levels were significantly higher only during the trough in LED-treated rats compared with LCD-treated rats. Furthermore, chronic alcohol treatment decreased thymus and spleen weight in both intact and ADX rats with CS replacement. These results indicate that continuous alcohol exposure (150-300 mg/dl) leads to a persistent activation of the HPA axis, particularly during the nadir of the diurnal rhythm.


Recent studies from our laboratory have demonstrated that chronic alcohol exposure (OT) pathways in the inhibitory control of solute ingestion, whether the solute is taken as food or as a concentrated NaCl solution. These data led us to investigate whether central OT may be similarly involved in the control of (ethanol) ingestion, since OT also affects ethanol intake. In the current study, male rats were pretreated with central injections of ricin A toxin conjugated to OT (AOT, 5 µg; i.c.v.) or the non-OT peptide, α-thrombin (2 µg; i.c.v.) 46 hours before the injections. Central injections of OT at either 1 µg (a specific receptor agonist) or 25 µg (a non-specific receptor agonist) resulted in significantly increased daily ethanol intakes compared to the controls at all ethanol concentrations tested (p<0.01). This marked ethanol preference in the AOT-pretreated rats produced significantly greater degrees of tolerance, as measured by lack of responsiveness to ethanol (EtOH) following 5, 10 and 15 sec i.c.v. (p<0.01). In addition, studies using potentiated OT injection in rats with pretreatment with central injections of AOT significantly increased daily ethanol intakes compared to the controls at all ethanol concentrations tested (p<0.01). These data indicate that central OT pathways are involved in the inhibition of ethanol intake. Our data suggest that ethanol is a potential therapeutic tool for the treatment of alcohol dependence.
659.1

**DISCRIMINATIVE AND AFFECTIVE STIMULUS PROPERTIES OF THE CALCIUM CHANNEL BLOCKER NIMODIPINE IN RATS.**

J. De Vis, M. de Jong* and R. de Bruijn. Institute for Neurobiology, Tropenwolke GmbH & Co. KG, Brain Research Ass. 156, 5163 College, FRG

The L-type calcium channel blocker nimodipine, a 1,4-dihydropyridine (DHP) derivative, is effective in reducing alcohol intake and preference in animal models of alcoholism. In order to gain more insight into possible mechanisms underlying the alcohol intake-suppressing effects of this drug, the discriminative and affective (rewarding/aversive) stimulus properties of nimodipine were evaluated in Wistar rats. It was found that the drug was equally effective in a standard all-delivered, lever-pressed reinforced discrimination (DD) procedure and, in subsequent generalization tests, inverted U-shaped dose-response curves were obtained with nimodipine with an effectiveness value of 9.0% (S.E.M. = 0.011). This response was generalized completely to (+)-nimodipine (again, an inverted U-shaped curve was obtained), but only partially to (-)-nimodipine. Although effective as a discriminative stimulus, the quality of this stimulus appears to be dissimilar to that of ethanol as it was found that rats trained to discriminate ethanol from saline in a similar procedure did not generalize to nimodipine. The latter finding was confirmed in a cross-familiarization conditioned taste aversion (CTA) procedure, an alternative method to reveal stimulus similarities between drugs. In addition to discriminative stimulus effects, nimodipine has also affective stimulus properties, as the compound was found to induce conditioned taste aversion (CTA), as well as, conditioned place preference (CPP). Racemic (+)-nimodipine were active in CTA and CPP, whereas (+)-nimodipine failed to produce either CTA or CPP; suggesting that, in contrast to the DD effects, the affective stimulus effects are mainly mediated by the activity of (+)-nimodipine. Because the alcohol intake- and preference-reducing effects of nimodipine reside mainly in the (+)-enantiomer, the mechanism involved in these effects may be related to the affective stimulus properties of nimodipine.

659.2

**THE USE OF A SUCCROSE-SUBSTITUTION PROCEDURE TO ESTABLISH ETHANOL AS A POSITIVE REINFORCER IN AN OPERANT RUNWAY.** C.I. Czechowski* and A. Ittenberg. Behavioral Pharmacology Lab., Dept. of Psychology, Univ. of California, Santa Barbara, CA 93106.

The reinforcing properties of self-administered ethanol were examined in nondeprived rats trained to traverse a straight-arm runway for the opportunity to drink an ethanol solution. Animals were tested once each day in the runway where each trial culminated in 6 min of access to a drinking bottle containing 5-10% ethanol. In order to ensure reliable and significant levels of ethanol consumption, we employed a modified version of a sucrose-substitution procedure originally described by Samson (1986) in an operant lever-pressing paradigm. In the present study, subjects initially ran the alley for access to high concentrations of sucrose (20% w/v) without ethanol. Over the course of days/trials the concentration of sucrose was gradually reduced while the concentration of ethanol was increased from 0 to 10%. This procedure successfully resulted in a mean ethanol intake of 6.65 g/kg (S.E.M. = 0.14) - a dose that rendered several animals ataxic and had clear behavioral/intoxication effects. These data suggest that the runway/sucrose-substitution procedure may provide a unique model for investigating the reinforcing properties of high doses of ethanol in nondeprived animals.

659.3


The objective of this study was to determine the effects of taste aversion training during adolescence on subsequent alcohol intake of the selectively bred alcohol-preferring P and high-alcohol drinking HAD lines of rats. Beginning at 30 days of age, male and female rat pups were fluid deprived 24 hrs before a 30 min access to a 10% (v/v) ethanol solution. Following ethanol exposure, the animals were given an i.p. injection of either saline or 0.15 M LiCl (10 ml/kg). A total of five training sessions were administered every other day with ad lib access to water on intervening training days. Twenty-four hrs after the last training trial, rats were given continuous free-choice between water and 10% ethanol for four weeks with food available ad lib. There were no obvious gender differences or line differences to the effects of taste aversion training. All LiCl-treated subjects avoided the usually preferred ethanol-habituated rats. For the entire four week test period (intake in week 4 was 0.6±0.1 g/kg/day; N=8), while saline-treated rats steadily increased their alcohol intake from 8±0.7 g/kg/day during week 4 (p<0.01 vs LiCl treated). Total volume of fluid intake did not differ between groups (39±4 ml/day for saline-treated and 39±5 ml/day for LiCl-treated group). Approximately 60% of the total fluid intake was ethanol for the saline-treated group, while the LiCl-treated rats this value was less than 3%. Rats in the saline and LiCl-treated groups gained weight at equivalent rates. These data suggest that early environmental intervention can prevent the onset of alcohol drinking in the selectively bred alcohol-preferring P and high-alcohol-drinking HAD lines of rats. (AA07462, AA08653, AA07611)

659.5

**CO-MORBID DEPRESSION, DRUG DEPENDENCE, AND ALCOHOLISM.**

Norman S. Miller, M.D.(1), Norman G. Hoffmann, Ph.D.(2), Mark S. Gold, M.D.(3) University of Illinois at Chicago(1), The University of Minnesota(2) and The University of Florida Brain Institute (3).

We examined the relationship between a lifetime diagnosis of major depression in 6535 patients with drug dependence and/or alcoholism. Evaluations were performed in a personal interview on admission (383 questions) and follow-up data was gathered by a structured telephone interview (110 questions) at 6 and 12 months. The most common diagnosis of a substance use disorder was alcohol (31.3%), followed by cannabis (19.7%) and then marijuana (12.3%). The rate of lifetime diagnosis of major depression was 43.7% and subclinical depression was 9.6%. Over half of the patients had two or more symptoms of depression and more than 35% described more symptoms. The rates of depression were significantly greater for females than males, for alcoholism than other drug dependencies. A lifetime diagnosis of major depression was significantly associated with poly-drug dependence.

**NUMBER & FREQUENCY OF DRUG USE BY DEPRESSION DIAGNOSIS.**

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*P < .0001

659.6

**NEURAL EXPRESSION OF C-FOS PROTEIN FOLLOWING AGGRESSIVE BEHAVIOR OR ETHANOL ADMINISTRATION IN MICE.** K. Bechter, G. Gunn, J. Murankaj, and F. von Saar* Division of Biological Sciences, University of Missouri-Columbia, Columbia, MO 65211.

We employed immunocytochemistry to detect the presence of c-fos protein in the brains of male wild mice one hour following i.p. treatment (20mg/kg) with 1.03 g/kg ethanol (EtOH) 21.67 g/kg EtOH 3.9% saline or 4.9% saline and an agnostic encounter with an unfamiliar conspecific male in a neutral arena 10 min. following injection. Mice subjected to an aggressive interaction or treated with 1.67 g/kg EtOH exhibited elevated c-fos expression in the anterior and olfactory cortices of the amygdala, medial preoptic area and entorhinal cortex relative to mice treated with 0.33 g/kg EtOH or saline. C-fos was also expressed in the piriform cortex of mice that fought and in the hippocampus of mice given the higher dose of EtOH. These results demonstrate c-fos activation in olfactory and limbic structures that have been implicated in the mediation of aggressive and sexual behaviors in other mammals. They also suggest that EtOH may affect mouse expression of c-fos gene expression in the olfactory-limbic pathway.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
The importance of temporal factors in the establishment and maintenance of ethanol withdrawal signs in the rat was questioned by a user who puts a tremor to his/her natural scale, and acoustic startle test. Ethanol dependence was invoked in naive male Wistar rats either by ethanol liquid diet administration for four exposures (n=16) for 14-17 days prior to withdrawal in both cases. The rats maintained in the ethanol vapor chambers were divided into 3 groups with target BALS of 120-140, 170-190, and 220-240mg/dL to determine the effects of BAL concentration on withdrawal measures. Both test groups had appropriate controls (n=8). Repeated measures ANOVA was performed on the data. Disturbance of the floor produced a piezoelastic response which was amplified, filtered, and stored in an IBM computer. Following each tremor trial, subjects were rated for withdrawal signs: verticromelic distal flexion reflex, irritability, tail stiffness, and abnormal body posture/ splayed gait. For acoustic startle, movements were detected by a piezoelectric accelerometer beneath the Plexiglas chamber, digitized and recorded on an IBM computer. Mixed trials with sound pulses of 105 and 120dB were included in each test session. Data analysis revealed that most withdrawal signs reached peak intensities between 8 and 12hr postwithdrawal. Most quantified signs of withdrawal were greater in the vapor chamber subjects than in the liquid diet subjects, probably reflecting higher chronic BA's in the former case. Results suggest that both liquid diet administration and chamber exposure produce quantifiable, time-dependent measures of ethanol withdrawal.

EXTRACELLULAR CONCENTRATIONS OF ETHANOL IN THE BRAIN FOLLOWING IP, IG OR CONSUMED ETHANOL IN RATS. K. Kilian, M. Nurm, and J.D. Sinclair, Biomedical Research Center, Alko Ltd., and Department of Zoology, University of Helsinki, Helsinki, Finland.

The distribution was examined by studying the extracellular levels of ethanol in the nucleus accumbens of the alcohol preferring AA (Alko Alcohol) and alcohol avoiding ANA (Alko Nonalcohol) rats with in vivo microdialysis. Samples for the assay of ethanol with head-space GC were collected from freely moving animals at 1 min intervals after administration of ethanol (1 g/kg) IP or IG, as well as during voluntary drinking of 10% (v/v) ethanol solution provided by the access to ethanol solution IP 30 min before daily. Tail blood was drawn every 5 min. The results show that there is a steep rise in brain ethanol concentration within minutes after IP injection, but there is no obvious difference between the two rat lines in the distribution of ethanol into the brain. The slopes revealed curvses from the same animals peaked much later. With IP administration, however, the time courses were similar for brain and tail-bloood ethanol. In the drinking study ethanol was found in the brain only a few minutes after the rats started to consume ethanol. The results and so far give no indication that the differential ethanol consumption by AA and ANA rats could be explained in terms of differences in the distribution of ethanol to the nucleus accumbens. Nevertheless, the observed level of brain ethanol after voluntary drinking is sufficiently large and rises quickly enough to provide positive reinforcement. Furthermore, the study shows that microdialysis coupled with head space GC gives detailed information of the brain ethanol curve in individual, freely moving animals after relatively small doses of ethanol.

LATENCY OF CONDITIONING TRIAL AND EXTENT OF CHRONIC CONDITIONING EFFECT EKMO-INDUCED CONDITIONED PLACE PREFERENCE AND AVERSION. G. G. Blassley, M. J. Lewis. Neurobehavioral Laboratory, Dept. of Psych., Temple U., Phila. PA 19122

The present study was designed to examine the effect of latency of conditioning trials and the extent of chronic conditioning upon a EKMO-induced place preference and aversion. Preliminary preference studies suggest that ethanol may gain its reinforcing effect only after a latency of one hour prior to exposure to the drug. Moreover, research from our lab has suggested that the latency of the conditioning trial to the time of administration is an important factor in EKMO's rewarding properties. Male albino rats were subjected to a paradigm of 1 hr stimulus (CS) and ip injections of varying doses of ethanol (0.25g/kg to 4g/kg). Subjects received one trial per day for four consecutive days and were given a free choice test on the fifth day. Each subject received three consecutive occurrences of this schedule. Conditioning sessions lasted 15 minutes and began at latencies ranging 15 or 30 minutes post-injection. Significant interactions between dose, extent of conditioning, and latency with preference and aversion were observed. It was suggested that EKMO has a synergy in rewarding and aversive properties depending upon these experimental variables.


We have continued our studies of the relationship between regional cerebral metabolism of glucose (CMRglc) and subjective responses to drugs of abuse. In order to focus on individual differences in behavioral effects, we have applied PET and parametric comparison procedures which enable us to character cerebral metabolic responses in two groups of 12 subjects each. Our previous PET and 1H MRS studies have demonstrated significant differences in regional metabolism between groups preferring and not preferring ethanol (E) placebo, and those preferring and not preferring the placebo, subjects were categorized into two equal groups: high liking (HL, mean difference of liking for ethanol compared to placebo +4.49, SD = 1.48) and low liking (LL, mean difference of liking for ethanol compared to placebo +4.53, SD = 1.29). PET data on CO2 uptake were correlated with anatomical MRIs, transformed to standard volumes based on the Talairach atlas, interpolated, and averaged across subjects for HL and LL. For each group the ethanol condition and placebo were compared using paired t-tests. No significant differences were observed.

CHRONIC INTRACEREBROVENTRICULAR ETHANOL ADMINISTRATION IMPAIRS NONSPATIAL MEMORY IN RATS. C.L. Weaver, L.M. Copley, R.S. Cottulett, and G.L. Dubner, Dept. of Psych., University of Michigan, Ann Arbor, Michigan, 48109.

Chronic IP injections of ethanol (ETOH) reduces behavioral flexibility by spatial learning tasks (Davenport et al., Behav. Neurosci., 103:1259-1266). We tested the effects of chronic ICV ETOH administration on spatial and nonspatial, working and reference memories on an eight-arm radial water maze (Hamm) task. Male rats were given sham (n=8) or chronic (21 days) ICV ETOH (20% in saline) or saline via mini-osmotic pumps. Both ETOH- or saline-treated rats could solve the spatial task at unoperated control levels. Rats receiving the ETOH made significantly more reference memory errors into channels that never had escape platforms) and working memory 'incorrect'(re-entries into channels previously visited within a trial that never had escape platforms) errors than both saline and unoperated controls during nonspatial testing. Our results indicate that chronic ICV ETOH can disrupt some aspects of both reference and working nonspatial memory. Supported by NSF Grant DUE 9352395 and Research Excellence Fund.
660.1
INTERACTIONS OF ETHANOL WITH NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS EXPRESSED IN XENOPUS OOCYTES. C.M. de Faria* and E.M. Mayer, Dept. of Pharmacology & Therapeutics, Univ. of Florida Col. of Med., Gainesville, FL 32610-0267.

While the correlation between smoking and drinking is very large, little is known about the biological factors which regulate the co-use of nicotine (Nic) and ethanol (EtOH). Previous evidence has suggested that sensitivity to these agents is genetically correlated because EtOH may modulate nicotinic acetylcholine receptor (nAChR) function by stabilizing nAChRs in the nonfunctional, desensitized form. Here we report that ethanol affects neuronal nAChRs in a subtype-selective fashion. Neuronal nAChRs subtypes (α3β2, α2β2 and α3β3) were expressed in Xenopus oocytes from cRNA and studied under two-electrode voltage clamp for responsiveness to bath-applied, Nic and EtOH. While EtOH displayed no direct agonist effects, it modulated the function of each of these receptor subtypes. At all receptor subtypes, there was a trend for cooperation of EtOH to enhance responses to Nic. In oocytes expressing α3β2 and α2β2 receptors, application of Nic 2.5 min after the start of a 5 min application of EtOH attenuated responses to AC applied 5 min following Nic (2.5 min after the end of the EtOH application). This effect was not seen at α3β3 receptors. Dose-response and time-course analyses are currently being conducted at these as well as at other nAChR subtypes. These results suggest that EtOH may display complex pharmacological properties at specific neuronal nAChR subtypes. Supported in part by training grant AA-07861. The cDNA clones were kindly provided by Dr. Jim Boulter of the Salk Institute.

660.2
ETHANOL MODULATION OF RECOMBINANT ACETYLCOLINE RECEPTOR CHANNELS EXPRESSED IN HEK-293 CELLS. A. Ravindran*, K. Masood and F. F. Weight, Laboratory of Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892.

The nicotinic acetylcholine receptor (AChR) is a ligand-gated ion channel located in the postsynaptic membranes of nerve, muscle and electric organ. The AChR from electric organ and muscle consist of four distinct subunits assembled into a α2β2γ (or αβγ) pentamer. The modulation of acetylcholine (ACh) induced current by ethanol was studied in HEK-293 cells transfected with mouse αβδ AChR subunit cDNAs. 40-48 hr after transfection whole-cell and out-side out patch clamp techniques were used to record ACh induced inward current from cells expressing AChR. ACh alone and in combination with ethanol (EtOH) were applied to the recorded cell and out-side out patch by a theta-tube rapid perfusion system that enabled complete exchange of solutions in 1-5 ms. ACh activated rapidly desensitizing inward currents with an EC50 of 20 μM. Co-application of EtOH (10-150 mM) potentiated, in a concentration-dependent manner, currents activated by 5 and 10 μM ACh. The potentiation was between 20-50% of the control response. With higher concentrations of ACh > 25 μM, EtOH seem to induce significant reduction in peak current amplitude. Further studies are being undertaken to elucidate the mechanism of ethanol modulation of AChR channels.

660.3

Ethanol (EtOH) has an extremely complex profile of actions on the CNS, and attention has increasingly focused on factors that create selective vulnerability of specific cell types. The hypothesis tested here was that EtOH effects in the somatosensory cortex may be related to specific neural attributes of membrane function, including electrical or biological signaling and morphological intracellular recording techniques combined with dye injections were used in vitro to investigate the actions of EtOH on synaptic transmission within layer V of the somatosensory cortex. Bath application of EtOH (1-100mM) revealed a large spectrum of effects on membrane parameters and postsynaptic potentials of cortical neurons. In some regular spiking neurons with a "bursting" type characteristics, EtOH (10mM) did not change input resistance and thebase but produced a blockade of accommodation. In this cell type, excitation postsynaptic potentials (EPSPs) evoked by electrical stimulation of white matter or adjacent layers were monophasic and very sensitive to the depressant action of EtOH. On the other hand, in a population of "bouffet" cells, the membrane and input resistance were altered by EtOH (10mM) in response to stimulation of the white matter, these neurons displayed a characteristic high-frequency response with early and late components. The late component was more strongly affected by EtOH. Neurons of the "intrinsinc bursting" type also had biphasic EPSPs whose late components were preferentially depressed by EtOH. Finally, in neurons with a regular spiking" neuronal circuitry, EtOH altered input resistance, blocked accommodation, decreased duration and amplitude of the slow afterhyperpolarization and reduced excitability effects in the late EPSP of the burst component. These results confirm that EtOH can exert variable effects on different neural elements of layer V neocortical circuits. The influence of EtOH on specific intracellular signaling pathways may underlie its effect on specific tasks. (Supported by NIDA DA 08405 to FMS and DA 08349 to AED).
660.5

EFFECTS OF CHRONIC ETHANOL EXPOSURE ON GABA$_A$ RECEPTOR $\beta_2$ and $\gamma_2$ SUBUNIT mRNA LEVELS IN RAT BRAIN. L. D. Dravet$^1$, D. R. Graven$^1$, and A. L. Morrow$^1$.

The expressions of mRNA encoding different subunits of the GABA$_A$ receptor were studied using RT-PCR method. In the brain of ethanol-exposed rats, the expressions of $\beta_2$ and $\gamma_2$ subunit mRNAs were affected, with a significant decrease in the $\beta_2$ subunit mRNA level and an increase in the $\gamma_2$ subunit mRNA level. These changes may contribute to the development of ethanol dependence.

660.6

AN IN SITU HYBRIDIZATION STUDY ON $\nu$-SUBUNIT mRNA OF GABA$_A$ RECEPTORS OF PENTOBARBITAL (PB) TOLERANT / DEPENDENT RAT BRAINS. T. Ito, T. Suzuki, D.K. Lim and X. H. Ho.*

The mRNA expression levels of the $\nu$-subunit of the GABA$_A$ receptor were studied in the brains of rats tolerant and dependent on pentobarbital. The results showed a significant increase in the mRNA levels in tolerant and dependent rats compared to the control group, indicating a possible role of the $\nu$-subunit in the development of tolerance and dependence.

660.7

GABAERGIC NEURONS IN THE EXTENDED AMYGDALA MODULATE ALCOHOL REINFORCEMENT. F. Hyvälä$^1$ and G. F. Koob$^1$.

The role of GABAergic neurons in the extended amygdala in the modulation of alcohol reinforcement was studied. The results showed that the activity of GABAergic neurons in the extended amygdala was increased in rats with a history of alcohol exposure, suggesting a potential role in the development of alcohol dependence.

660.8

CHANGES IN GABAERGIC SYNAPSES OF DENTATE GRANULE CELLS DURING CHRONIC ETHANOL TREATMENT. E. Filley$^1$, H. Eason$^1$, K. Buntzem$^1$, P. Schuck$^1$.

The synaptic changes in the dentate granule cells of rats exposed to chronic ethanol were studied using immunohistochemistry. The results showed a decrease in the number of GABAergic synapses, suggesting a potential role of synaptic changes in the development of ethanol dependence.

660.9

INTRA-STRATUM ADMINISTRATION OF FLUMAZENIL (FL) TO DIZEPAM (DZ) DEPENDENT RATS. E. P. Winter$^1$, J. A. Sloman$^1$, J. Ying$^1$.

The effects of intra-stratum administration of flumazenil on the behavior of dizepam-dependent rats were studied. The results showed a decrease in the sedative and hypothermic effects of dizepam, suggesting a potential role of flumazenil in the modulation of dizepam-induced effects.

660.10

ETHANOL INCREASES TAURINE BUT NOT GLUTAMATE NOR GABA IN THE NUCLEUS ACCUMBENS USING MICRODIALYSIS. A. Dachhour$^1$, K. Quertermont$^1$, Ph. Durbin$^2$ and Ph. De Witte$^2$.

The changes in the levels of taurine, glutamate, and GABA in the nucleus accumbens of rats treated with chronic ethanol were studied using microdialysis. The results showed an increase in the levels of taurine and a decrease in the levels of glutamate and GABA, suggesting a potential role of taurine in the modulation of ethanol-induced changes.

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DECREASE OF PAIRED-PULSE INHIBITION IN CA1 AREA OF HIPPOCAMPAL SLICES OF CHRONIC INTERMITTENT ETHANOL TREATED RATS: M.E. Kandy, L. Spiegelman, D.M. Rapp, and R.W. Olsen. Dept. of Pharmacology, UCLA School of Medicine, School of Dentistry, Los Angeles.

Chronic intermittent ethanol (CIE)-treated rats showed severe withdrawal signs including a persistent kindling-like decrease in the pentetrazol (PTE) seizure threshold (Kokka et al., Alcohol Clin. Exp. Res., 525-531, 1993). To understand the cellular mechanism underlying this plasticity, the involvement of GABA receptors, the target of ethanol and PTE pharmacological effects, was studied utilizing biochemical and electrophysiological functional assays. We observed a significant decrease in muscimol (100 μM)-induced GABA efflux in hippocampal slices from CIE rats. Paired-pulse inhibition, which is considered to be predominantly due to GABA-mediated recurrent inhibition, was significantly (p<0.005) decreased in the CA1 area of hippocampal slices from CIE rats measured 2 days post-ethanol. This study suggests that a hypofunction of GABA receptors in the hippocampus may play a key role in kindling-like decrease in the seizure threshold in the CIE rat model of alcohol dependence. Supported by AA07640.


This study was designed to determine the effects of alcohol upon: (1) defensive rage behavior (DB) elicited from the medullary hypothalamus (MH), and (2) MH facilitation of DR elicited from the doral midbrain periaqueductal gray (PAG). Experiment I: peripheral administration of alcohol (0.02, 0.5 and 1.0 g/kg, i.p.) reduced latency responses in a dose and time dependent manner, the maximal effect occurring at 60 min, postinjection, at the highest dose level. Experiment II: peripheral administration of alcohol at the same dose levels markedly enhanced MH facilitation of DR elicited from the PAG in a dose and time dependent manner. Experiment III: microinjections of the NMDA receptor antagonist, AP-7, into PAG-DR sites blocked MH facilitation of DR elicited from the PAG. These results indicate that: (1) alcohol administration facilitates the occurrence of DR; and (2) the excitatory effects of alcohol upon rage may be manifest through the MH-PAG pathway which involves NMDA receptors. [Supported by NIH Grant NS 07941-24]
ROLE OF PROTEIN KINASE C IN ETHANOL INDUCED INHIBITION OF METABOTROPIC GLUTAMATE RECEPTOR FUNCTION IN PRIMARY CULTURES OF ASTROCYTES. T.L. Smith* and M.S. Birrick. Research Service (151), Dept. of Veterans Affairs Med. Ctr., Tucson, AZ 85723

Chronic ethanol (E) exposure selectively inhibits metabotropic-glutamate receptor (mGluR) activation in astrocytes (Smith, Alcohol, in press 1994). Because this receptor system is highly sensitive to modulation by protein kinase C (PKC), the aim of the present study was to determine whether the inhibiting effect of E could be reversed in the presence of staurosporine, a PKC antagonist. Astrocytes were cultured in DMEM with 5% FCS in 35 mm dishes in the absence of 100 mM Fe for 4 days. In some studies, control and E treated cells were co-incubated with 10 mM staurosporine for 3 days or with 2 μM calphostin C 10 mins prior to addition of agonists. After a 24 hr. preincubation with [3H]inositol (1μCi/mL), astrocytes were stimulated with 1S, 1R -ACPD and subsequent [3H]InsP3 determined. Chronic E significantly inhibited the InsP3 response to 1S, 3R -ACPD. Moreover, exposure for 10 min to 10μM phorbol ester (PMA) further inhibited the response in E treated cells, indicating that the effects of E and PKC activation were additive. The results suggest that the inhibitory effect of chronic E is not mediated primarily by PKC. (Supported by a research grant from the Dept. of Veterans Affairs, Washington, DC).

MODULATORY EFFECTS OF ACUTE ETHANOL ON METABOTROPIC GLUTAMATE RESPONSES IN CULTURED PURKINJE NEURONS. J.G. Netzeband* and D. L. Grudel. Dept. Neuropharmacology and the Alcohol Research Center, The Scripps Research Institute, La Jolla, CA 92037.

Of the many actions contributed to ethanol in the brain, little attention has been given to possible interactions with metabotropic glutamate receptors that may be involved in synaptic transmission and plasticity. To this end, we modified organotypic cultures were prepared from embryonic day 20 rat cerebella and extracellular recordings were made from Purkinje neurons at 21-37 days in culture (37 °C). Metabotropic glutamate responses were induced by pressure ejection of (a) 300 μM (1S,3R)-1-aminocyclopentane-1,3-di-carboxylic acid ([1S,3R]-ACPD) or (b) 5 μM quisqualate (50 μM DNGQ was used to block the ionotropic component of quisqualate-evoked responses). Both (1S,3R)-ACPD and quisqualate produced biphasic responses consisting of an initial brief excitatory phase (5-20 s) followed by a prolonged inhibitory phase (10 s ≥ 2.5 min). These agents also induced increases in the appearance of burst-like activity. In the presence of 66 mM ethanol, (1S,3R)-ACPD-mediated responses exhibited a decrease in the magnitude of the excitatory phase and an increase of the total response duration, with no change in the induction of burst activity compared to controls. In contrast, 66 mM ethanol had much different effects on quisqualate-mediated responses. For quisqualate, ethanol decreased the total response duration and the induction of burst activity, but had no effect on the magnitude of excitation. These studies suggest a complex interaction between the effects of ethanol and metabotropic glutamate receptors in cultured Purkinje neurons. Supported by ARC 8420 and AA 0765.


Embryonic exposure to ethanol (ETHO) often produces motor dysfunction consisting of poor balance, altered gait and decreased tendril (stretch) reflexes. To determine if alterations in the development of serotonergic somata and fibers in the spinal cord contribute to these symptoms, chick embryos were exposed to ETHO. Surprisingly, the development of serotonergic neurons and fibers was studied with immunohistochimical techniques using a polyclonal antisera to serotonin (5HT). Groups of chick embryos were treated with 10% ETHO in sterile Tyrode's w/v as compared to control embryos. Immunolabelings of either 0.1 μM or 0.2 μM w/v (medium dose) or 0.275-0.352 μM w/v (high dose). Control embryos were treated with equivalent volumes of sterile Tyrode's. The ETHO was administered as a single beginning day 1 (E1) and continued until the animals were sacrificed. Serotonin immunoreactivity [5HT-IR] was examined in the first 3 lumbosacral spinal cord segments in animals sacrificed on E10, E12 and E14. At all stages examined, the density of 5HT-IR fibers was less in both ETHO-treated groups than in control groups. Camera lucida drawings of 5HT-IR neurones were utilized for number of primary dendrites (those originating directly from the soma) and secondary dendrites (any dendrite originating from a dendrite). At all stages examined, the number of secondary dendrites was significantly less in both ETHO-treated groups as compared to controls. There were significantly fewer primary dendrites in both ETHO-treated groups at E12 and E14 and in the embryos treated with the high ETHO-dose at E10. These ETHO-induced alterations in the serotonergic system may be involved in the motor dysfunction observed after embryonic ETHO exposure. (Supported by AA02055).


IMPACT OF ETHANOL ON DEVELOPMENT OF ORGANOYTIC CULTURES FROM NEOCORTEX AND SPINAL CORD. D.L. Davies* and B. Mendelson. Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205.

Development of the CNS is adversely influenced by exposure to ethanol. As part of an endeavor to assess the effects of ethanol on neural development in the absence of confounding systemic variables, organotypic cultures were stereotopically evaluated following ethanol exposure. Organotypic cultures were prepared from the neocortex of 4-day-old postnatal rats and the spinal cords of embryonic day 10 chicks. Groups of cultures from both sources were incubated in medium containing 0%, 0.25%, 0.5% or 1% (v/v) ethanol; cultures were harvested for evaluation after 1, 3, 6 and 9 days of in vitro ethanol exposure. At these timepoints, both control and ethanol exposed cultures from neocortex and spinal cord retained features of the fetal structures. In these cultures, a detailed set of astrocytes identified by immunolocalization of glial fibrillary acidic protein. Throughout the culture interval, serotonergic neurons in the spinal cord were immunocytochemically labeled. The exposure of embryonic cultures to ethanol exposed cultures. The somatic and dendritic arbors of motoneurons and particular classes of interneurons were labeled by placement of small crystals of DiI at specific locations on the fixed spinal cord. In the neocortex, ethanol exposed has exhibited cultures exhibited smaller dimensions than control cultures; this was more evident at the high (0.5%) ethanol concentration and may reflect lower cell viability. These studies suggest the utility of organotypic cultures as alternative models for delineation of the direct effects of ethanol on development and cellular interactions in the CNS. Supported by NIH grants AA07145 (DL) and AA09205 (BM).
NO REDUCTION OF SPONTANEOUSLY ACTIVE MESOLIMBIC DOPAMINERGIC NEURONS IN ETHANOL-WITHDRAWN RATS. [661.5] (D.C. Mab, J. Stuel, M. Basile*, E. M. Dole, J. Wagger, F. E. Livin, and R. P. Palmier, Dept. of Neurology, Pharmacology & Pathology, Univ. of Miami School of Medicine, Miami, FL, 33101 and Dept. Psychiatry and Centre for Human Genetics, McGill University, Montreal, Canada) The binding characteristics of dopamine synaptic markers were studied in four different test systems: alcohol withdrawal, pre-drinking, alcohol-pretreatment, and short-term alcohol abstinence. We have used radioligand binding and autoradiography to quantify and map the status of D1 dopamine receptors in ethanol-consumption and alcohol-preferred monkeys. Our analysis demonstrates that the number of D1 receptors labeled with [HHSCH 23900 is elevated over limbic structures of the basal forebrain and striatum. Saturation binding analysis demonstrates a high density of D1 receptors in the nucleus accumbens, but not in the prefrontal cortex. This finding suggests that ethanol withdrawal could lead to increased D1 receptor expression, which could potentially contribute to the increased alcohol consumption observed in ethanol-withdrawn animals. The implications of these findings for understanding the mechanisms underlying alcohol withdrawal and relapse are discussed.

DEFICIENCIES IN DOPAMINE AND SEROTONIN CNS SYSTEMS ASSOCIATED WITH HIGH ALCOHOL PREFERENCES. [661.6] (L.M. McDermott, E. Hamlet, J. Sokoloff, and T. L. G. Dept. of Psychiatry, Univ. of Miami, Med. & Med., Univ. Indiana Sch. Med. and VA Med. Ctr., Indianapolis, IN 46202-4860). The hypothesis that high alcohol preference is associated with abnormalities in certain dopamine (DA) and serotonin (5-HT) CNS systems was investigated. In one study, the contents of DA and 5-HT were determined in 4 CNS regions of adult male rats in the F2 generation selectively bred alcohol-prefering P and alcohol non-prefering NP lines. Rats with the highest (N) and lowest (N) alcohol intakes (6.3±0.3 g 0.24±0.2 g/day, p=0.001) were compared. The results show that there is a reduction in the number of spontaneously active dopamine neurons from ethanol-withdrawal in a D2-neuroesterase rat. Since there is a possibility that the use of anesthetics could be a confounding factor in the number of spontaneously active dopamine neurons as evidenced by a calculated index unchanged between ethanol-withdrawal and saline-treated control rats, in chronic alcohol-treated rats, we repeated the experiments in anesthetized and chloral hydrate anesthetized rats. In conclusion, the results suggest that there is indeed a reduction in the number of spontaneously active mesolimbic DA neurons recorded from chloral hydrate anesthetized rats but this is confirmed to the anesthetized rats (D2). This finding does not provide evidence for the idea of impaired dopamine function in ethanol-withdrawn rats.

INVOLVEMENT OF THE DOPAMINE AXIS IN A VERTET MONKEY MODEL OF ALCOHOL ABUSE. [661.7] (R.M. Palermo, D. Miles, F. E. Livin, and S. N. Young*, Dept. of Psychiatry, McGill Univ. Sch. Med., Montreal, Que, Canada, H3A 1A1 & Dept Neurology, U Miami Sch. Med, Miami, FL) Approximately 15% of vertet monkeys voluntarily consume large quantities (≥ 5 g/kg/day) of unrestrained ethanol (Enlin et al., 1990). Several convergent lines of evidence suggest that dopaminergic neuronal transporters may differ between Alc:preferring (AA07462) and Alc:avoiding (AA09562) animals. As noted previously (Marchant et al., 1993), the density of DA transporters is high in abstinent Alc:monkeys and reduced in Alc:apomorphine-treated animals. In this study, we measured the effect of ethanol withdrawal on the expression of DRD1, DRD2, DRD3, and DRD4 mRNA levels in the mesolimbic dopaminergic system of naive and ethanol-withdrawn rats. The results show that ethanol withdrawal leads to a significant reduction in the expression of DRD1, DRD2, DRD3, and DRD4 mRNA levels in the mesolimbic dopaminergic system, suggesting that ethanol withdrawal leads to a significant reduction in the number of spontaneously active mesolimbic DA neurons as evidenced by a calculated index unchanged between ethanol-withdrawal and saline-treated control rats, in chronic alcohol-treated rats, we repeated the experiments in anesthetized and chloral hydrate anesthetized rats. In conclusion, the results suggest that there is indeed a reduction in the number of spontaneously active mesolimbic DA neurons recorded from chloral hydrate anesthetized rats but this is confirmed to the anesthetized rats (D2). This finding does not provide evidence for the idea of impaired dopamine function in ethanol-withdrawn rats.
661.11

CLOMIPRAMINE ALTERS THE RESPONSE OF VENTRAL TEGMENTAL AREA (VTA) NEURONES TO ETHER-INJECTED EXCITATION. R.D. Trinjooyil and M.S. Rodehe. Dept. Physiology and Biophysics, University of Illinois at Chicago, Chicago 60612.

Exitation of dopamine neurones of the ventral tegmental area (VTA) may be a critical factor for the rewarding effect of ethanol (EtOH). EtOH increases the firing rate of VTA neurones in vivo and in vitro and the potency of EtOH to excite VTA neurones is increased by serotonin (5-HT). Drugs which block the reuptake of 5-HT, like zimelidine and clomipramine (CLOM), have been used clinically to decrease the firing rate of VTA neurones. Our objective was to test whether clomipramine, at concentrations which block 5-HT reuptake, alters the response of VTA neurones to EtOH, as we have shown for 5-HT.

Coronal brain slices from the VTA were prepared from young adult F344 rats. Concentrations of EtOH (40 - 120 mM) were tested in the absence and presence of CLOM (125 mM to 2 mM). All neurones (n = 21) studied had electrophysiological characteristics typical of dopaminergic neurones and were excited by EtOH in a concentration-dependent manner. Clomipramine itself had little or no effect on the firing rate of VTA neurones. In the presence of 500 mM CLOM, EtOH-induced excitations were approximately doubled; with CLOM, the mean excitatory effect of EtOH increased from 10% to 20% for 40 mM EtOH, and increased from 34% to 58% for 120 mM EtOH. This potentiation is consistent with the action of CLOM to block 5-HT reuptake, and with our earlier findings that 5-HT potentiates the excitatory effects of EtOH on VTA neurones. At higher clomipramine concentrations (1 - 2 mM), there was no significant enhancement of EtOH-induced excitation. These higher concentrations are associated with reuptake blockade of norepinephrine and dopamine, which may obscure the effect of enhanced 5-HT availability. Grant Support: PHS AA01925; Alcoholic Beverage Medical Research Foundation.

661.12

CHRONIC ETHANOL EXPOSURE ALTERS DOPAMINE D2 RECEPTOR mRNA EXPRESSION IN NUCLEUS ACCUMBENS IN RATS. B.A. Blanchard & P.K. Roland. Dept. of Pathology & Anatomical Sciences, Univ. of Missouri School of Medicine, Columbia, MO 65212.

Brain dopamine (DA) systems are believed to mediate the reinforcing properties of drugs of abuse, including ethanol (EtOH). Chronic EtOH exposure in rats has been shown to alter DA D2 receptor populations, with the direction of change possibly dependent on the dose of EtOH and the period of exposure. The present study examined effects of chronic EtOH exposure on expression of the DA D2 receptor gene in the terminal regions of the mesolimbic and nigrostriatal DA pathways: the nucleus accumbens and striatum. Adult female Long-Evans rats were exposed to EtOH via liquid diet (35% EtOH-derived calories) for periods of either two or six weeks. Following EtOH exposure, DA D2 receptor mRNA content in the core and shell regions of the nucleus accumbens, and medial and lateral regions of the striatum was examined using in situ hybridization histochemistry. After two weeks of EtOH exposure, there were no significant changes in DA D2 receptor gene expression in any region of either structure. However, six weeks of EtOH exposure produced a significant decrease (approximately 40% below control levels) in DA D2 receptor mRNA content in the core region of the nucleus accumbens. There were no significant changes in this region in either the core or shell regions of the striatum. The findings suggest that chronic EtOH exposure may alter DA receptor populations by altering gene expression in regions believed to be important in drug reward.

661.13


We previously found that ethanol-induced depressions of cerebellar Purkinje neurons in Sprague-Dawley rats are mediated by a GABA, mechanism, but that ethanol alone only potentiates GABA responses on 20% of these cells under concomitantly applied with a d-adrenergic agonist. Timolol, a d-adrenergic antagonist, not only blocks these ethanol-induced potentiations of GABA, but also antagonizes ethanol-induced depressions on 20% of these neurones as well. These data may suggest that synaptically-generated norepinephrine alters the responsiveness of this subpopulation of Purkinje neurones to ethanol actions under the conditions of our experiments. In the present study, we investigated these ethanol actions in two lines of rats which have been selectively bred for high (HAS) and low (LAS) GABA sensitivity. We recorded single cerebellar Purkinje neurones from an anterior barrel of a multibarrel microinjection chamber, with localised application of drugs from other barrels of the same pipette in urethane-anesthetized rats. Similar to Sprague-Dawley rats, we found that timolol reduced ethanol-induced depressions in 16% of these neurones. On 62% of the HAS neurones, however, the ethanol responses were antagonized by timolol application. Based on this observation, we hypothesized that ethanol would more frequently potentiates GABA-induced depressions in LAS than in HAS rats. Indeed, we observed this phenomenon in 69% of LAS neurones studied, but only in 20% of HAS neurones. These data suggest that there is a difference in the interaction of ethanol with GABA mechanisms between HAS and LAS rats which may be regulated by the activity of endogenous d-adrenergic mechanisms.

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661.14


We previously found that ethanol-induced depressions of cerebellar Purkinje neurons in Sprague-Dawley rats are mediated by a GABA, mechanism, but that ethanol alone only potentiates GABA responses on 20% of these cells unless concomitantly applied with a d-adrenergic agonist. In the present study we found that timolol, a d-adrenergic antagonist, not only blocks ethanol-induced GABA, potentiations, but also antagonizes ethanol-induced depressions on 20% of these neurones as well. These data may suggest that synaptically-generated norepinephrine alters the responsiveness of this subpopulation of Purkinje neurones to ethanol actions under the conditions of our experiments. Supporting this conclusion, we found that phenolamine, a agent that facilitates noradrenergic synaptic function in the cerebellum, reversibly potentiates the depressant effects of locally applied ethanol. Furthermore, we investigated the influence of acute morphine withdrawal on the sensitivity of Purkinje neurons to ethanol-induced depressions, a condition in which there is increased noradrenergic synaptic input from the locus coeruleus. We found that after 7 days of chronic morphine treatment, the systemic injection of naloxone, a morphine antagonist that precipitates withdrawal in chronically morphine-treated animals, enhanced the depressant responses of cerebellar Purkinje neurones to local applications of ethanol and PCP. These data suggest that endogenous d-adrenergic mechanisms regulate ethanol actions in the cerebellum.

(Supported by USPHS grant AA05915. MP is supported by ADAMHA Research Scientist Development Award AA01052.)

661.15

SUPPRESSIVE EFFECTS OF ALCOHOL UPON PREDATORY ATTACK BEHAVIOR IN THE CAT ARE BLOCKED BY A SUBSTANCE P, NK, RECEPTOR ANTAGONIST: M.D. Shaltiel* Y.C. Han, L. Polchery, D. Benjamin, and A. Sigel. Laboratory of Limbic System Behavior, Department of Anatomy, New Jersey Medical School, Graduate School of Biomedical Sciences, UMDNJ, Newark, New Jersey 07103, and Center for Alcohol Studies, Rutgers University, Piscataway, New Jersey, 08855.

This study was designed to determine the effects of alcohol upon quiet biting "predatory" attack behavior (QBA) elicited from the lateral hypothalamic area (LH) and the mechanism within the medial hypothalamus (MH) involved in suppression of this response. Experiments I: peripheral administration of alcohol (0.02, 0.5 and 1.0 g/kg, I.P.) resulted in a dose and time dependent suppression of QBA in which the maximal effect (42% suppression, observed 60 min., post injection, when tested with 5% alcohol. Experiment II: peripheral administration of alcohol at the same dose levels also increased medial amygdaloid (MEA)-induced suppression of QBA in a dose and time dependent manner. Experiments III: microinfiltration of Substance P (SP)-NK, antagonist, CP 96,345, into MH blocked alcohol-induced enhancement of the suppressive effects of ME stimulation upon the LH. These results suggest the following conclusions: (1) that alcohol suppresses QBA; (2) that the underlying mechanism for suppression of this response may involve agonistic-like effects of alcohol upon SP receptors within the MH, which receive direct inputs from an SP pathway originating in the ME. [Supported by H.F. Guggenheim Foundation and NIH grant NS07941-24].
EFFECTS OF ETHANOL ON MIDDLE LATENCY AUDITORY EVOKED POTENTIALS IN THE RAT. H. Miyazato, R.D. Skinner* and E. Garcia-Bailon. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

The P1 middle latency auditory evoked potential in the human peak at 50-60 msec latency and REM sleep, but absent during slow wave sleep; i.e., it is present during periods of cortical desynchronization. Recently, we described the presence of the P13 potential (peak 11-15 msec) in the rat which commonly occurs before the P1 potential and wave A in the cat, the feline equivalent of the P1 (Skinner et al., 1993). The present study was undertaken to determine the effects of ethanol on the behavior of the rat P13 potential.

Under barbiturate anesthesia, cortical leads were implanted at the vertex and over the temporal cortex. An intragastric tube was prepared and routed to exit the dorsal neck. Auditory stimuli (intensity 103 dB, rate 0.2 Hz) evoked P13 potentials referenced to a frontal screw were recorded and averaged from male rats following intragastric ethanol administration (0.5, 1.5, or 5 g/kg, 48% in water). Following a 0.5 g/kg dose of ethanol there was no effect on the P13 potential. However, at a 5 g/kg dose of ethanol the P13 potential was reduced to 60% at 5 min and then recovered by 15 min. Larger doses of ethanol (3 and 5 g/kg) reduced the P13 potential to 30% and 20%, respectively. The P13 potential recovered after 30 min following the 3 g/kg dose, and after 75 min following the 5 g/kg dose. Similar recording of the averaged auditory evoked responses (P3) over the auditory cortex showed only a slight effect on this potential at all ethanol doses tested.

Our results suggest that ethanol reduces the amplitude of the P13 potential and delays its recovery in a dose-dependent manner. Thus, the behavior of this potential indicates that ethanol decreases the ability of the reticular activating system to generate a normal arousal response.

Supported by USPHS grant NS26246.

INHIBITION OF CALCIUM ENTRY BY CADMIUM CHANGES ETHANOL INHIBITION INTO EXCITATION IN RAT LOCUS COERULEUS NEURONS. S.S. Osmanovic* and S.A. Shepherd. Department of Physiology and Biophysics, University of Illinois at Chicago, College of Medicine, Chicago, IL 60612-7342.

We have previously shown that ethanol can inhibit the spontaneous firing of rat locus coeruleus (LC) neurons. In the present study, intracellular recording in completely submerged brain slices from Fisher 344 rats was used to study the involvement of Ca2+ entry in the ethanol-induced inhibition of LC neurons. Ethanol was tested before and after application of the calcium channel blocker Co2+ or low Ca2+/high Mg2+ media. In 7 LC neurons which showed ethanol-induced inhibition, the mean spontaneous firing rate in control media was 0.72 ± 0.18 Hz (S.E.M.) and 0.28 ± 0.09 Hz after bath application of 100 mM ethanol. The mean ethanol-induced inhibition of firing, calculated when each spike was compared to its own control, was 49 ± 4% (S.E.M.)

The ethanol-induced inhibition of spontaneous firing was associated with no change in resting membrane potential (n=5) or membrane hyperpolarization of 2.4 mV (n=2). Effects of ethanol were lost after superfusion with media containing 2 mM (100 - 500 mM) Ca2+ for 15 - 25 min. In the presence of Co2+, ethanol caused membrane depolarization and increased the firing rate in all 7 neurons tested; the mean depolarization was 7.7 ± 0.36 mV and 9 ± 3.6 mV, respectively. The effect of ethanol in Co2+ was significantly different from the control as determined by a paired t-test (p < 0.05). Ethanol-induced inhibition was also changed to excitation when the Ca2+ concentration in the media was reduced from 2.4 to 0.25 mM and the Mg2+ concentrations increased from 1.5 to 10 mM (n=5). These data indicate that ethanol-induced inhibition of spontaneous firing in LC neurons is dependent on Ca2+ entry. Another previously reported action of ethanol on LC neurons is reduction of action potential duration. This action of ethanol was not affected by Co2+ indicating that ethanol effects on spontaneous firing and action potential duration are mediated by separate mechanisms.

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Repeated withdrawals from chronic ethanol consumption have been linked to an exacerbation of withdrawal symptoms, suggesting that multiple exposures and withdrawals from ethanol lead to a progression of the symptoms that is not apparent with continuous ethanol administration. Previous research has shown that both ultrasonic vocalizations and the expression of fos-like proteins are increased by repeated ethanol administrations. In the present experiment, rats given repeated withdrawals from ethanol, rats given the same amount of ethanol continuously, and control animals were compared on the basis of ultrasonic vocalizations in response to air puff and fos-like immunoreactivity (Fos-LI). Behavioral testing occurred seven to eight hours following ethanol withdrawal on the final day of the experiment. The results showed that both withdrawal groups spent more time vocalizing in comparison to the control animals, while the repeated withdrawal group demonstrated increased vocalization in comparison to the continuous ethanol group. Immunohistochemical analysis of c-fos activity showed a similar trend for specific brain regions. In particular, Fos-LI in the medial prefrontal cortex, ventral lateral orbital cortex, piriform cortex, retrosplenial cortex, locus coeruleus, and tenia tecta was enhanced more by repeated withdrawals than a single withdrawal. These data support the hypothesis that multiple ethanol exposure/withdrawal cycles result in a more severe withdrawal syndrome than a single ethanol withdrawal. AA-08042, NS26555, & HD07201.

663.5 EXPRESSION OF HEAT SHOCK PROTEINS FOLLOWING EXPOSURE TO CHRONIC ETHANOL. E.L. Otto-Reeves, J. Mark Sherman, Clayton M. Pickering and John D. Lane.* Department of Pharmacology, UNTISHC-F, 3500 Camp Bowie Blvd, FW, TX 76107 USA.

Heat shock proteins are induced in cultured cells when the culture medium contains 5% ethanol. Patients with a history of chronic alcohol exposure show high levels of heat shock proteins (HSP). In this experiment, male Fisher 344 rats were taught to orally self-administer ethanol in an FR schedule, using sucrose fading. These rats were the only low responding group for ethanol alone. Rats were fed a liquid diet containing ethanol, then retested over a range of ethanol concentrations. Ethanol intake increased over levels prior to liquid diet. Rats were then fed the liquid diet for 2 weeks. Tissues from heart, liver, kidney, gastrointesstial tract, skeletal muscle and multiple brain regions were harvested and in situ hybridization was performed using oligonucleotide probes specific for either inducible or constitutive 70kD heat shock proteins. Both inducible and constitutive mRNAs were found in brain. In brain, the highest levels of both inducible and constitutive mRNAs were found in the cerebellar granular layer, hippocampus, dentate gyus and hypothalamic nuclei. In kidney, the inducible probe showed uniform distribution; however, the constitutive form showed a specific pattern with highest levels of mRNA in the medulla. All other organs showed more uniform distribution of both the inducible and constitutive mRNAs. In regions showing a statistically significant difference, levels of inducible mRNAs were greater than constitutive in both ethanol and control rats.

663.6 ENHANCED (Na+/K)-ATPase EXPRESSION IN MOUSE BRAIN AFTER CHRONIC ETHANOL ADMINISTRATION. Y.M. Choo, J.B. Womack, A.Y. Sun, Dept. of Pharmacology, University of Missouri, Columbia, MO 65212.

It is the general hypothesis that the primary mode of action of ethanol is the alteration of membrane structure and function including the conformation of receptors and ion channels essential for neurotransmission and signal transduction. (Na+/K)-ATPase is of interest because of its major role in neuronal homeostasis and membrane dependence. In the past we have demonstrated the inhibitory effect of ethanol on (Na+/K)-ATPase in vitro, and chronic administration of ethanol has led to an increase in the specific activity of this enzyme. However, the issue of whether ethanol affects (Na+/K)-ATPase under physiological conditions remains unsettled. In this study, adult mice were treated with a daily dose of 5 g/kg of ethanol for 28 days. The RNA was isolated from brain samples and probed for the (Na+/K)-ATPase mRNA using Northern blot analysis. We have found an increased (Na+/K)-ATPase in the chronically treated alcohol group as compared with controls. This result was further substantiated by the increased protein phosphorylation of this enzyme after chronic ethanol administration. Thus we have demonstrated that ethanol may directly or indirectly affect (Na+/K)-ATPase in situ, leading to the increased synthesis of this enzyme through adaptive mechanisms. (Supported by NIH Grant AA02054).


Alcohol-prefering P rats exhibit less exploratory behavior (despair)* in a forced swim test than -nonpreferring NIH rats (Godfrey et al., Soc. Neurosci. Abs. 18543, 1992) Overstreet et al. J. N. N. 1988). However, the AA alcohol drinking rats exhibit more immobility than drinking rats in a swim test (Korpi et al., 1986). The present study compared swimming for 5 min in rats that low drinking LAD rats on a swim test and examined the effects of desipramine (10 or 20 mg/kg). Adult male HAD and Dose were placed in a round tub filled with water (21°C). The rats were unable to escape from the tub. Behavior was videotaped for 10 min on each of two consecutive days and scored for time spent immobile. HAD rats were more immobile (P<0.05) than LAD rats (10 vs. 1 sec mean (SEM), respectively). Desipramine attenuated (p<0.05) the time spent immobile in LAD but not in HAD rats which contrasted with the low responsiveness seen in previous both P and NP rats with greater attenuation. The swim test is not associated with a genetic predisposition to alcohol preference. Attenuation of immobility by desipramine is seen in both alcohol-nonpreferring (NP) and LAD lines but not in both -preferring lines. (AA0611, AA08555).

663.8 REPEATED ETHANOL WITHDRAWAL EXPERIENCE DIFFERENTIALLY INFLUENCES WITHDRAWAL-RELATED SEIZURES AND ANXIETY RESPONSES IN MICE. H.C. Becker, K.G. Fernandez, and R.T. Weatherston. VA Medical Center and Medical University of South Carolina, Charleston, SC 29412.

We previously demonstrated an exacerbation of withdrawal seizures in adult CDH mice that expressed multiple cycles of ethanol (EOH) withdrawal in comparison to mice withdrawn from EOH at a single time, even when total EOH exposure is equated across groups. This study was designed to examine whether similar results may be obtained in another mouse strain, and with another symptom of withdrawal (anxiety). Adult male NIH Swiss mice were exposed to EOH vapor prior to withdrawal. A multiple withdrawal (MW) group received 3 cycles of 16 hr of EOH vapor separated by 8 hr periods of abstinence; a continuously exposed (CE) group received the same total amount of EOH (48 hr) interrupted; a single withdrawal (SW) group received a single 16 hr bout of EOH exposure; and controls received no EOH exposure. Following the final withdrawal cycle, mice were tested for handling-induced convulsions (HIC) or performance on an elevated plus-maze. Blood EOH levels upon final withdrawal were similar among EOH-exposed groups (160-200 mg%). The groups did not differ in % time on or % entries into open arms of the plus-maze. 24 hr post-withdrawal. Total arm entries were depressed in the MW and CE groups at 8 hrs, but not at 24 hrs. In contrast, severity of withdrawal seizures was significantly greater in the MW group in comparison to other groups. Mean area under the 24 hr HIC curve was 213±3.2 vs. 106±4.7, 133±3.0, and 10±2.3 (M±SE). These results suggest that in this mouse strain, repeated EOH withdrawal exacerbates the severity of subsequent withdrawal seizures, but does not influence anxiety, as measured in the plus-maze test. Other mouse strains are being used to continue to explore this research question. Supported by VA Medical Research Service and NIAAA.
**662.10**

**ETHANOL PARTIALLY NORMALIZES VESTIBULAR PRONATION OF PONTINE-DAMAGED RATS BUT DISRUPTS THAT OF UNDAMAGED RATS.**


Acute or chronic ethanol (EOT; 5-40%) does not abolish excessive forward locomotion in rats with damage of the nucleus reticularis tegmenti pontis (NRTP). Although EOT interacts with motor activity in intact rats, it can improve some motor functions of NRTP-damaged rats. Pronation of the head and torso from a supine position in the air using an integrated sequence of movements is an index of neural integrity that is compromised by basal ganglia dysfunction. In NRTP-damaged rats, pronation is disrupted by a change in form from lateral to ventralflexed righting. 11 female and 6 male Long-Evans hooded rats were lesioned electrolytically in the NRTP (1mA anodal current/15s bilaterally, n=8) or used as controls (n=9). Testing was done over 50 days during availability of tap water (TW), TW+25% sucrose (SU), or 5-30% EOTH in TW+SU in water bottles. A challenge dose (CD) (30%W/DOT) was administered i.p. after the withdrawal. Righting was tested by holding the animals in a supine position at 3 cm above a 7cm thick pillow and releasing them (lateral form=1, ventroflexion=0; 4 trials/animal/substance/dose). On TW, NRTP-damaged rats showed more ventralflexed than lateral righting (x=5) and undamaged rats showed lateral righting exclusively (x=1). NRTP-damaged rats normalized slightly on SU (x=.6), but unoperated rats began to ventroflex (x=.7). At each level of EOTH, NRTP-damaged rats showed more lateral righting than on TW (x=.68 for 5-30%EOTH & 75 at 30%CD; range=.58-.75 vs. TW=5), and unoperated rats showed less (x=.76 for 5-30%EOTH & 88 at 30%CD). The results indicate that EOTH can improve vestibular function in NRTP-damaged rats, and further support an appetitive role for EOTH in the presence of pontine damage.

**662.11**

**IN UTERO ETHANOL EXPOSURE RETARDS GROWTH & ALTERS MORPHOLOGY OF CORTICAL CULTURES: GM1 REVERSES EFFECTS.**


Ethinol: a dental neuronoxin, affects the cerebral membrane physico-chemical properties. Its multiple actions in the developing CNS are complex, affecting embryogenesis, cell migration, differentiation & synaptogenesis. In a prior study using a model for the fetal alcohol effect, the neuroprotective lipid GM1 reduced fatty acid ethyl ester accumulations in rat fetuses exposed to ethanol in utero (207&8 & 13.14A) (Hungund et al., 1993). This study was initiated to further describe the in utero effects of ethanol, and, the capacity of GM1 treatment to ameliorate such changes. Sprague-Dawley CVP dams were exposed to ethanol (23 g/kg i.p. supra). GM1 ganglioside (10mg/kg/m) was given 24hrs & 1hr prior to each ethanol exposure. Cortical cultures were derived from GD15&8 fetuses. Since GM1 is highly localized on the plasma membrane outer surface of CNS cells, we have used it as a marker molecule to assess cell integrity. We used cholea toxin/vinltoxin fluorescence immunohistochemical to localize GM1. Cultures were stained at 4, 8, 14 days after plating. Results indicate that the brief in utero exposures to ethanol affected cell growth and morphology. We observed a marked retardation in cell development and arborization as early as 24hrs after plating. Also, there was a reduced number of viable cells (24hrs thru 14dats). Control cells showed continuous staining for GM1 along outer plasma membranes. Ethanol exposed cells exhibited decreased and discontinuous membrane staining for GM1. This reduced staining is associated with the loss of membrane integrity (Laev et al., 1993). These in utero ethanol induced abnormalities are pathologically diminished in those cultures derived from fetuses of dams treated with GM1.

**662.12**

**EFFECT OF ALCOHOL EXPOSURE DURING DEVELOPMENT ON THE AMYGDALA REGION.**

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The only perinatal period in the rat is roughly equivalent to the third trimester in humans with respect to birth. Exposure to alcohol during this period has been shown to decrease the DNA concentration in the amygdala region of male rats at both 45 and 90 days of age. Female rats showed no effects. The decrease in DNA concentration suggests that there is an alcohol-induced decrease in cell number in the amygdala region, although the DNA concentration does not differentiate among cell types. In order to continue our investigation of the amygdala region, rats of both sexes were artificially reared and exposed to 5 g/kg/day of ethanol, artificially reared and not exposed to ethanol, or reared normally with dams. Rats were weaned at 21 days of age and housed with a same sex conspecific. At adulthood (130 days), the rats were deeply anesthetized with sodium pentobarbital and perfused intracardially with saline followed by 10% formaldehyde. The brains were removed and post-fixed in 10% formaldehyde for at least a week. The brains were cut into 40 um sections, mounted on slides, stained Luxol Fast Blue, and then counterstained with cresyl violet. A section at the level of the stria terminalis was identified and the area of the amygdala region was measured. Among female rats, no differences were found in the areal measurements of the amygdala region. (Supported by NIAAA Grant AA08080 to S.J.K.)
INHIBITORY EFFECT OF THE CALCIUM ANTAGONIST ISRADIPINE ON NICOTINE INTERVENTIOUS SELF-ADMINISTRATION IN DRUG-NAIVE RATS. W. Pratt*, M.C. Martelotto, A. Kozmin, E. Zvartau. 1 Department of Neuroscience, University of Cagliari, Italy. 2 Department of Pharmacology, Pavlov Medical Institute, St. Petersburg, Russia.

Several reports indicate that nicotine positive reinforcing effect is associated with a depression of the mesolimbic dopaminergic system. Thus, in common with other drugs of abuse, such as morphine and cocaine, nicotine increases dopamine release in rat striatum and nucleus accumbens. We have recently shown that isradipine, a L-type dihydropyridine Ca2+ channel antagonist, inhibits the reinforcing properties of cocaine and morphine both in rats and mice. This effect is likely due to an inhibition of cocaine- and morphine-induced dopamine release. Here we show that isradipine dose-dependently and stereospecifically inhibits nicotine intravenous (i.v.) self-administration in drug-naive mice. In fact, nicotine induces a dose dependent i.v. self-administration response in drug-naive mice. Pretreatment with (+)-isradipine significantly inhibits nicotine i.v. self-administration in drug-naive mice. Furthermore, testing of both the isomers of isradipine reveals that only pretreatment with (+)-isradipine is active in inhibiting nicotine i.v. self-administration in drug naive mice, while pretreatment with the (-)isomer is completely inactive. The possibility that the isradipine inhibitory effect might be related to an inhibition of nicotine-induced dopamine release seems to be very likely. Furthermore, these results might provide an indication for new therapeutic strategies in tobacco addiction.


Prefrontal areas display most prominent decreases in immediate-early gene expression during amphetamine withdrawal. We employed antisense technology in vivo, to assess whether prefrontal c-fos may play a role in behavioral correlates of rat amphetamine withdrawal.

Phosphorothioated antisense 18-mers aimed at the translation start site of the c-fos mRNA display a half-life of about 3 hrs, in contrast to 15-30 min for phosphodiester. Intracranial medial prefrontal cortical injections of 250nm antisense, but not sense, phosphorothioated oligos, block c-fos mRNA translation, while exerting variable and inconsistent effects on c-fos mRNA levels. During c-fos translational blockade, animals display marked reductions in linear and repetitive locomotor behavior when exposed to a novel environment. No change in locomotor activity is evident in animals accustomed to the activity monitor. These changes closely mimic those recorded during amphetamine withdrawal and suggest that prefrontal c-fos may play a role in the chain of neurobiological events responsible for amphetamine withdrawal-induced behavioral alterations.


Psychostimulant drugs such as amphetamine can induce dependence syndromes and presumably long-term changes in the CNS. Altered expression of genes may provide possible biochemical contributions to these drugs induced long-term CNS changes. We have analyzed alterations in gene expression in brains of rats sacrificed 4 hours after injection of d-amphetamine (7.5 mg/kg, i.p.), using differential display PCR followed by differential hybridization. More than 20 bands which were changed after drug administration could be identified after amplification with two primer sets. Some were represented in all brain regions analyzed, and some showed region-specific patterns. Sequence analysis of differentially-displayed products revealed high homologies with known genes. One product revealed 97% homology with the reported sequence of a calcium activable calmodulin-dependent phosphatase, calcinulin. Northern analysis showed that striatal calcinulin mRNA increased by 40% after d-amphetamine treatment. The second cDNA encoded a transducin-like factor. Hybridizing mRNA showed marked, approximately 10-fold, increases in striatum with changes in cortex and thalamus also notable. Products of genes acutely regulated by d-amphetamine are candidates for participation in drug-induced long-term changes in the CNS.

BEHAVIORAL SENSITIZATION TO AMPHETAMINE IS CORRELATED WITH A GREATER INCREASE IN C-FOS mRNA LEVELS IN THE STRIATUM OF RATS. K. Rivero*, T. A. Wise*, T. D. Paolodi and S. Rivero*. 1 School of Pharmacy, 1Mol. Endo. Lab., CHUL, Laval university, Quebec and 2CSBN, Dept. of Psychol., Concordia Univ., Montreal.

Most studies exploring the neural basis of behavioral sensitization converge on the mesocambudrom dopaminergic pathway. The mechanisms underlying this phenomenon have been less clear, however. More complex and recent data suggest the participation of additional neurotransmitters. To better understand the mechanism of behavioral sensitization, we have performed in situ hybridization analysis of c-fos mRNA in the brain of animals sensitized to amphetamine. Male Long-Evans rats received daily injections of saline or amphetamine (1 mg/kg) for 10 consecutive days. One week later they were given an acute challenge injection of saline or amphetamine (0.5 mg/kg). The animals were sacrificed 45 min or 3 h after the last injection and their brains were collected for further in situ hybridization analysis of c-fos mRNA. Amphetamine (1mg/kg) increased locomotor activity and the increase was progressively greater as animals received repeated injections. The challenge injection of 0.5mg/kg produced a significantly greater increase in locomotor activity and c-fos mRNA levels. Characterization of the neurons in which c-fos responses to amphetamine are increased in sensitized animals is presently under investigations. Supported by the MRC.
663.7


Previous studies have demonstrated increased locomotor activity and immediate-early gene expression in the cerebellum following the administration of psychostimulants. In the present study, immunohistochemical techniques were used to assess the pattern of Fos-like immunoreactivity (FLI) in the cerebellum following acute, systemic administration of amphetamine and cocaine. Amphetamine (1.5 mg/kg, i.p.) increased locomotor which could be blocked by pretreatment with the D1 dopamine receptor antagonist SCH 23390 (1 mg/kg). Within the cerebellum, amphetamine elicited a dose-dependent increase in FLI that was markedly attenuated by SCH 23390 pretreatment. In contrast, SCH 23390 pretreatment abolished the dose-dependent increase in locomotor activity and FLI following cocaine (10, 20 mg/kg). Psychostimulant-induced FLI was restricted to the granule cell layer within each of the medullar cerebellar lobules (IX-X) and distributed in dense clusters extending from the molecular layer to the Purkinje cell layer. Studies assessing the effects of acute, systemic caffeine administration on FLI in the cerebellum are in progress. Preliminary results indicate that caffeine (15 mg/kg) produces a somewhat different pattern of cerebellar FLI with greater involvement of the molecular layer than seen following amphetamine and cocaine.

663.8

NBOX INHIBITS AMPHETAMINE-INDUCED LOCOMOTOR ACTIVITY BUT NOT C-FOS INDUCTION. A. Dalia*, N.J. Urelesky and L.J. Wallace*, College of Pharmacy, Ohio State University, Columbus, OH 43210.

Administration of amphetamine activates the dopaminergic and the AMPA subtype of glutamate receptors as well as induces c-fos protein in the nucleus accumbens. A role for basal levels of c-fos in expression of locomotor activity is derived from the observation that administration of c-fos antisense oligonucleotide into the nucleus accumbens 5 hours before amphetamine blocked the locomotor response. Therefore, a hypothesis that the basal activation of glutamatergic receptors, increases in c-fos protein, and stimulation of locomotor activity was explored. A behaviorally relevant dose of amphetamine (1 mg/kg, p) elicited a small increase in c-fos protein and stimulated locomotor activity. Pretreatment with NBOX (23-dihydroxy-6-nitro-7-sulfamoylbenzo(F)-quinolinaxidine) (30 mg/kg, p), an AMPA receptor antagonist, had no effect on amphetamine-induced increase in c-fos but stimulated locomotor activity. NBOX alone had no effect on c-fos levels or locomotor activity. MK-801 (0.1 mg/kg, sc), an NMDA receptor antagonist, did not increase c-fos but stimulated locomotor activity. These data suggest that induction of c-fos is not correlated with stimulation of locomotor activity. (Supported by DA07722 and DA06776).

663.9

NBOX INHIBITS LOCOMOTOR ACTIVITY INDUCED BY APOMORPHINE IN DRUG-NAIVE BUT NOT SENSITIZED RATS. P. Leudtke* and L.J. Wallace, Ohio State University, College of Pharmacy, Columbus, OH 43210.

The development of sensitization to apomorphine (apo), cocaine, and amphetamine is considered to be mediated by changes in dopamine neurotransmission. Since the acute effects of these agents involve activation of the AMPA receptor subtypes of glutamate receptors, the present study investigates whether the sensitized state is also characterized by a change in glutamate neurotransmission. On day 0, male rats were conditioned to the test cages. At each treatment day they were habituated for one hour. Pretreatment (i.p. and s.c.) and distance travelled measured for two hours by automated image analysis. In drug-naive rats, NBOX (10 mg/kg), an AMPA antagonist, attenuated locomotor activity induced by apo (1 mg/kg) to 45%. However, when pretreated with apo (5 mg/kg) every third day during 13 days, the locomotor activity induced by apo (1 mg/kg) was increased, while NBOX only attenuated to 83%. Preliminary results indicate that sensitization to cocaine and amphetamine also decreases the inhibitory effect of NBOX. These results indicate that sensitization and a change in response to NBOX occurs simultaneously. Additional studies will elucidate if these changes are a part of sensitization and further describe the nature of the alterations. (Supported by a gift of NBOX from Novo Nordisk A/S and grant number DA07722).

663.10

COMPETITIVE AND NONCOMPETITIVE NMDA-RECEPTOR ANTAGONISTS BLOCK NICOTINE-INDUCED DOPAMINE OVERFLOW. F. Museo* and A. Pert, Biological Psychiatry Branch, Intramural Research Program, National Institute of Mental Health, Bethesda, MD 20892.

The psychomotor stimulant effects of nicotine are believed to be due, at least in part, to the activation of a dopaminergic substrate; one dopamine (DA)-containing region that appears to be involved is the nucleus accumbens (nAcB). We previously reported that the systemic or intra-nAcB administration of nicotine elicited DA overflow in the nucleus accumbens. In the present experiment we tested this hypothesis using two NMDA-receptor antagonists: the noncompetitive antagonist MK-801 and the competitive antagonist AP-5. Male, Sprague-Dawley rats were anaesthetized with chloral hydrate and a microdialysis probe was surgically positioned in the nAcB. All pharmacological treatments were administered through the probe and dialysate samples were collected and analyzed for DA content. Nicotine (100 μM), on its own, increased nAcB DA overflow by approximately 50% in the intra-nAcB administration of either the noncompetitive antagonist MK-801 (100 μM) or the competitive antagonist AP-5 (100 μM) prior to and during the administration of nicotine (100 μM) significantly reduced the magnitude of this effect. It appears that the blockade of glutamatergic transmission is sufficient to disrupt the actions of nicotine on DA overflow in the nAcB.
663.13

BOTH TYPICAL AND ATYPICAL NEUROLEPTICS BLOCK PHENCYCLIDINE (PCP)-INDUCED STRIATAL AND BEHAVIORAL EXCITATION IN FREELY MOVING RATS. I. M. White*, C. G. Flor, J. Speziale, and G. V. Revel. Program in Neural Science and Dept. of Psychology, Indiana University, Bloomington, IN 47405.

The striatum plays a critical role in the behavioral excitation induced by amphetamine and other indirect dopamine agonists. In freely moving rats, amphetamine typically increases the activity of striatal neurons, with this effect being blocked by D2 dopamine antagonists, which also attenuate amphetamine-induced behavioral effects. Phencyclidine (PCP), a non-competitive NMDA antagonist with little or no affinity for dopamine receptors, induces a similar pattern of behavioral excitation, but little information is available on the striatal mechanisms underlying this effect. In the present series of experiments, we assessed the effects of PCP (1.0 - 5.0 mg/kg) on single-unit activity in the striatum of awake, behaving rats. Like amphetamine, PCP routinely increased striatal activity, and this effect was reversed by subsequent injection of either haloperidol (0.1 mg/kg) or clozapine (20.0 mg/kg), typical and atypical neuroleptics, respectively. To the extent that neuroleptics act primarily as dopamine antagonists, our results suggest a striatal dopamine-NMDA interaction in the behavioral effects of PCP.

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663.15

AMPHETAMINE- AND COCAINE-INDUCED FOS IN THE RAT STRIATUM DEPENDS ON D2 DOPAMINE RECEPTOR ACTIVATION. D.N. Bunks* and R.F. Marshall. Department of Psychology, University of California, Irvine, CA 92717.

Amphetamine or cocaine injection causes expression of the immediate-early gene c-fos in the striatum. Previous studies have shown that dopamine D1 receptor activation is necessary for this effect, but have not established a consistent role for D2 receptors. We have investigated the involvement of D2 receptors in indirect agonist-induced striatal Fos-like immunoreactivity using the selective D2 antagonist eticlopride. Rats received eticlopride (0.5 mg/kg) or saline 30 min before receiving amphetamine (5 mg/kg), cocaine (40 mg/kg), or saline. All injections were IP. Two to four hours after the second injection, the rats were perfused with 4% paraformaldehyde and their brains sectioned at 40 µm through the striatum for immunocytochemistry. Eticlopride treatment caused Fos expression by itself, but also decreased Fos expression in the central striatum due to amphetamine or cocaine by 90% and 85%, respectively. In striatogniral neurons, identified by labeling with the retrograde tracer Fluorogold injected into the substantia nigra pars reticulata, the blockade of stimulation-induced Fos-like immunofluorescence by eticlopride was nearly complete, with decreases of 98% for amphetamine and 94% for cocaine. In striatogniral neurons, the D2 antagonist alone induced minimal Fos. We conclude that activation of both D1 and D2 receptors classes by dopamine agonists is necessary for induction of Fos in the striatogniral cells of normal rats. These results provide an important parallel to behavioral and electrophysiological work that also demonstrates D1/D2 interdependence in the control of normal basal ganglia functions.

663.17


We studied self-stimulation in rats with chronic cannulae implanted into the substantia nigra pars reticulata. Using clozapine (15 mg/kg, m=14) or saline (m=14) was injected IP 15 min prior to self-stimulation testing. Electrode placements were independently verified by two different researchers and compared with drug effects. The distance between the electrode placement and the brain midline was measured. The correlation of this distance with stimulant-induced facilitation of self-stimulation was r=+.43, p<.004. Inspection of the data suggested that stimulation sites closest to substantia nigra pars compacta showed the least stimulant-induced facilitation of self-stimulation. The correlation between drug effect and distance of electrode from the substantia nigra was r=+.44, p=.003. The findings indicate that amphetamine and cocaine may differentially influence self-stimulation obtained from the A9 and A10 regions.

This project was supported by NIDA grant # DA 04483.

663.14

THE ROLE OF DOPAMINE D3 RECEPTORS IN BEHAVIORAL SENSITIZATION, SENSITIVITY TO AMPHETAMINE, AND DOPAMINE SYNTHESIS. P.H. Robitsek*, F. Malizia, R. Melander, and D. Kuhar. Departments of Psychology, Morehead State Univ., Morehead, KY 40351, and University of Kentucky, Lexington, KY 40506.

Behavioral sensitization to repeated treatments of the mixed D2/D3 dopamine (DA) receptor agonist apomorphine (APD) can be blocked by the D2 antagonist SCH 23390 but not D2 antagonists. Further, APD-induced basalis DA synthesis as does the D2/D3 agonist quinupride. This effect is blocked by the D2 antagonist eticlopride. Moreover, sensitization to APD appears to be D2 mediated D2 receptors may be involved in the increased DA synthesis. The role of DA D3 receptor activation in locomotor activity, sensitivity to APD and basalis DA synthesis were investigated in two experiments. D3 selective agonist 7-OH-DPAT was administered at 24 hour intervals for 15 consecutive days (0.5 - 1.5 mg/kg). Following each injection locomotor activity was recorded for 20 min. On day 11 in experiment 1, all rats received a challenge injection of APD (1.0 mg/kg) and their locomotor activity was recorded. On all rats were injected with the DOPA decarboxylase inhibitor NSD 1015 (100 mg/kg). After 30 min the brains were removed and DA synthesis was assessed by measuring DOPA accumulation in the extracted tissue. Inoculation of activity was initially inhibited at the higher doses (0.1, 1.5 mg/kg) but increased to control levels by day 11. The low dose (0.01 mg/kg) also inhibited activity initially but increased to control levels at the higher doses. This inhibition increased with repeated treatment. No increase in sensitivity to APD was observed in experiment 2. The D3 treatment did not significantly increase baseline DA synthesis. These findings suggest that the development of behavioral sensitization to APD, and the accompanying increase in basalis DA synthesis, is not mediated by D3 receptor activation. (Supported by grants from the Kentucky/MSF SPCCN committee and Morehead State University).

663.16

EFFECT OF INTRAVERNAL SELF-ADMINISTRATION OF d- and L-AMPHETAMINE ON NUCLEUS ACCUMBENS (NAc) DOPAMINE LEVELS. K. Leeb* and R.A. Wise. Center for Studies in Behavioral Neurology, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

Rats given limited access to a variety of stimulant drugs self-administer these drugs with very regular inter-response times (Pickens and Thompson, 1968; Yokel and Pickens, 1974). To date, the variables responsible for maintaining such a regular pattern of responding for drug have not been fully established. Because amphetamine increases extracellular levels of dopamine (DA) in the NAc we investigated whether fluctuations in dopamine correlated with responding for drug. Male Long-Evans rats with chronic intraventricular cannulae and intra-accumbens guide cannulae were trained to lever-press for infusions of either 0.25 mg/kg d-AMPH or 0.75 mg/kg L-AMPH in 4h daily sessions. Once stable self-administration was established, rats underwent microdialysis during self-administration. Dialyse samples were taken every 5 min and frozen for subsequent determination of DA concentration using HPLC with electrochemical detection. Self-administered AMPH caused rapid elevation of DA levels to 300-500% of baseline, and these elevations were maintained, more or less, for the remainder of the session. However, there were phasic fluctuations in DA, superimposed on the tonic elevations, that were time-locked to lever-presses and the consequent drug injections; DA levels usually increased after each injection and fell prior to the next response. Thus the initiation of a drug-seeking response (e.g., a lever-press) was triggered in the absence of dopamine depletion and, indeed, long before dopamine concentration fell significantly toward basal levels.

663.18

RESERPINE ABOLISHES AMPHETAMINE-INDUCED DOPAMINE RELEASE IN VENTRAL MIDBRAIN NEURALN CULTURE. C. St. Remy, S. Rayport and D. Sulzer*. Depts Psychiatry, Neurology, Anatomy & Cell Biology; Ctr Neurobehavior & Behavior; Columbia Univ; Dept Neurorpathology, NYS Psychiatric Institute, NY 10032.

Amphetamine-induced (AMPH-induced) dopamine (DA) release has been suggested to occur at plasma membrane uptake transporters or at synaptic vesicles. Since several studies have reported that reserpine, which depletes vesicular DA, does not reduce AMPH-induced DA release, action at the vesicular level has been controversial. However, in these studies reserpine was administered in vitro to 24 h prior to AMPH, providing sufficient time for increased DA synthesis or other time-dependent effects. To circumvent these issues we used postnatal ventral midbrain DA neuron cultures, in which stimulation-dependent, AMPH-induced DA release and total cellular DA content can be reliably and concurrently measured. We found that AMPH (100 nM) reduced stimulation-dependent (40 mM KC1 for 3 min) DA release by 60% after 15 min and by 90% after 60 min. At a higher concentration, reserpine (10 µM) reduced AMPH-independent DA release to undetectable levels and total cellular DA by 95% at 90 min. In control cultures, 10 µM AMPH induced a 25% increase in extracellular DA, while following reserpine (10 µM) AMPH-induced only a 3% increase. Strikingly, AMPH released over 3-fold more DA from untreated cultures than the total DA content of reserpinized cultures. Together with in vivo microdialysis studies (Sulzer et al., Soc stenroni 4, 1993), this indicates that AMPH releases DA primarily from the vesicular pool, consistent with the weak base model of AMPH action (Sulzer et al., J Neurochem, 1995).
AGE-DEPENDENT PERSISTENCE OF FENFLURAMINE EFFECTS ON MONOAMINERGIC NEURONS IN REAGGREGATE TISSUE CULTURE. L. Won, P.C. Hoffmann and A. Haller, Dept. of Pharmacological & Physiological Sciences, University of Chicago, Chicago, IL 60637.

The three-dimensional reaggregate tissue culture system provides a useful approach to examine the effects of drug exposure on the subsequent development of monoaminergic neurons. Utilizing this system, we have previously demonstrated that exposure of 15 day old reaggregated tissue cultures developing monoaminergic cells (equivalent to postnatal day 7 neurons), to 10^{-5} M (±) fenfluramine (FEN) for 7 days resulted in significant reductions of reaggregate dopamine (DA)- and serotonin (SERT) levels and cell deficit persisted for 3 weeks following cessation of drug exposure. In order to examine the possible age-dependency of such effects, 2 month old reaggregate tissue cultures containing mesencephalon and corpus striatum cells (equivalent in development to adult neurons) were exposed to 10^{-5} M (±) FEN for 7 days and then were allowed to recover in drug-free medium for 3 weeks. At the end of the 7-day exposure to FEN, DA and 5-HT levels in reaggregates were significantly depressed to 35% and 69% of control levels, respectively. By the end of the 3-week recovery period, DA levels in FEN-treated reaggregates had attained similar values to those in control cultures whereas 5-HT levels in reaggregates exposed to FEN remained significantly depressed (52% of control). Thus, in reaggregate culture, both DA and 5-HT neurons are susceptible to the persistent effects of FEN during early development. With mature neurons, such long-lasting effects are only observed on 5-HT neurons as is seen in the intact adult brain. Supported by MH42194.

ADMINISTRATION OF PERTUSSIS TOXIN IN THE VENTRAL TEGMENTAL AREA DOES NOT ENHANCE THE LOCROTOM STIMULATION SIMULATION PRODUCED BY D-AMPHETAMINE. Brezina, D., Wulff, D., Wulff, D., N. Urasbey, L.J. Wallace, Ohio State University, College of Pharmacy, Division of Pharmacology, Columbus, Ohio 43210.

Readministration of amphetamine, cocaine, or apomorphine produces a progressively enhanced locomotor response. Recently it has been found that the bilateral administration of pertussis toxin (PTX) into the ventral tegmental area (VTA), the location of the mouse brain's ascending mesolimbic dopamine neurons, produces an enhanced locomotor response to amphetamine and cocaine similar to the changes seen in animals sensitized to these drugs. The current results compared to a previous study demonstrate the development of sensitization to drugs that act presynaptically to increase dopamine transmission. In the present study we investigated whether administration of PTX into the VTA produces a sensitized locomotor response to apomorphine, which directly activates dopamine receptors. Rats were injected into the VTA with PTX (0.5 μg, 0.5 μl per side) or vehicle, and 14 to 21 days later, the locomotor responses to apomorphine (0.5 μg/kg, pu) or apomorphine (1 and 5 μg/kg, s.c.) were determined. Animals treated previously with PTX showed a markedly enhanced locomotor response to apomorphine compared to vehicle-treated animals. However, the response of PTX-treated animals to either dose of apomorphine was not significantly greater than that of vehicle pretreated controls. Thus, while pretreatment with PTX in the VTA mimics some of the components of sensitization produced by drugs that indirectly stimulate central dopamine systems, it is not sufficient to produce a sensitized response to a directly acting dopamine agonist. (Supported by DAO7722 and DAO8776)

CORRELATIONS OF NOVELTY AND AMPHETAMINE INDUCED LOCOMOTION IN RATS WITH MICRODIALYSIS MEASUREMENTS OF DA NEUROTRANSPORTER FUNCTION. A. Delgado and C.W. Bradbury, Yale Univ. Sch. of Med., Dept. of Psychiatry and the West Haven VA Hospital, West Haven, CT 06516.

Using a circular runway locomotor chamber, we have been investigating biochemical correlates of novelty induced locomotion. Previous work by Piazza, et al. indicates this is predictive of amphetamine induced locomotion and of amphetamine self-administration. Novelty and amphetamine induced locomotion were assessed for 20 days, separated by at least 10 days. Individual locomotor scores from the two sessions were highly correlated. Following amphetamine challenge, animals were implanted with microdialysis guide cannulae overlying the VTA of the right hemisphere (DA) and the intra-nucleus accumbens (5-HT). The resulting needle was infused, and the intra-nucleus accumbens was monitored by microdialysis probes which were left in place overnight for experiments the following day. The first study examined the correlation of locomotor scores (both novelty and amphetamine induced) with basal DA (as assessed by triphenyl tetrazolium probe recovery and peak DA levels following local infusion (via the microdialysis probe) of 3 μM amphetamine for one minute collection period. Significant correlations were seen between novelty (as assessed by triphenyl tetrazolium probe recovery and peak DA following local infusion expressed either as an absolute level, or as a percent of basal). Basal levels also did not correlate with peak levels. In a separate study, rats were injected with amphetamine and basal DA, but not triphenyl tetrazolium probe recovery. Justice's group has demonstrated that in vivo recovery is primarily a function of DA uptake. In this group, a nonsignificant trend (p = 0.10) toward a correlation was seen between in vivo recovery and locomotion induced novelty, but not with amphetamine induced novelty. Supported by DA 80073, DA 80227, The Yale VA Alcoholism Research Center, and a NARSAD Young Investigator Award to CBW.

THE STIMULATION OF LOCOMOTION BY MK-801 IS MEDIATED BY THE ACTIVATION OF MESO-LIMBIC DOPAMINERGIC NEURONS. T. Narayanan, A. Datta, L. Wallace, N. Urasbey, Ohio State University, College of Pharmacy, Division of Pharmacology, Columbus, Ohio 43210.

Low doses of MK-801 (0.05-0.25 mg/kg), a noncompetitive antagonist of NMDA receptors, stimulate a coordinated locomotor activity by enhancing dopamine (DA) neurotransmission in the nucleus accumbens (NAC). This stimulation may be related to the ability of this drug to increase the firing rate of meso-limbic DA neurons. To test this hypothesis, we assessed the locomotor response to MK-801 in rats after administration of baclofen, a GABA-B agonist that inhibits the firing rate of meso-limbic DA neurons, into the ventral tegmental area (VTA). MK-801 (0.1 mg/kg, ip) stimulated locomotor activity, and this effect was inhibited by baclofen (0.15 mg/kg per side) injected into the VTA. This inhibitory effect appears to be related to an action on meso-limbic DA neurons, since baclofen, administered into the VTA or nucleus accumbens, was ineffective in blocking locomotor activity, and this effect was blocked by baclofen (0.15 mg/kg per side) injected into the NAC. Thus, the locomotor stimulant effect of systemic MK-801 may be mediated by an action of this drug in the VTA, producing an increase in the activity of DA neurons, resulting in an enhancement of DA neurotransmission in the NAC. Supported by grant DA07722.

REDUCED RESPONSIVENESS OF D1 RECEPTOR DEFICIENT MICE TO THE EFFECTS OF D1 SELECTIVE AGONISTS. ANTAGONISTS AND D-AMPHETAMINE IN TESTS OF LOCOMOTOR ACTIVITY AND CATECHOLPSY. L. H. Goldberg, M. Xu, I. Polis, C. Heyser, S. Tongeawa and J. F. Kenob. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037 and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139.

Dopamine (D1)-receptor deficient mice were created through introduction of a deletion in the gene encoding the D1 dopamine receptor by homologous recombination. ES cells carrying the deletion were injected into mouse blastocysts to generate chimeric mice which were then bred to obtain homozygous D1 knockout mice. The complete absence of D1 receptors was verified by genomic Southern analysis. In wild-type mice, the D1 agonist SKF81297 (1.2, 4.8, and 7.4 mg/kg, s.c.) produced a dose-dependent increase in locomotor activity compared to saline. SKF81297 had no effect in D1-deficient mice. The D1 antagonist SCH23390 (10, 30, μg/kg, s.c.) produced a dose-dependent decrease in locomotor activity in wild-type mice, but not in the D1-deficient mice. SCH23390 (0.05, 0.1, 0.2 mg/kg, s.c.) also produced dose-dependent increases in the wild-type mice, measured as percent of time immobile in an elevated ring procedure, but was ineffective in the D1-deficient mice. Interestingly, the D1-deficient mice exhibited a marked attenuation of the locomotor stimulation produced by d-amphetamine (1.3 mg/kg, s.c.), an indirect dopamine agonist, compared to wild-type mice. These results demonstrate that D1 receptor ligands are ineffective in D1 receptor deficient mice in behavioral assays of motor activity, and support the further use of these mice to investigate the relationship between D1 and D2 receptors in the behavioral actions of psychostimulant drugs and drug reinforcement.

SELECTIVE INVOLVEMENT OF DOPAMINE D1 RECEPTORS OF THE VTA IN THE BEHAVIORAL SENSITIZATION INDUCED BY INTRA-VTA AMPHETAMINE INJECTIONS. Y. Bisogno, L. Simons, L. Ma Moi and M. Caldec. Unit INSERM U259, rue Camille Saint Saëns 33077 Bordeaux, France.

In agreement with previous studies, we demonstrated the complete independence of the neurobiological substrates responsible for the induction of the behavioral sensitization to amphetamine (AMP). Using a local approach, we showed that AMPH injected at the level of the dopamine (DA) cell bodies (0, 1, 2.5, 5 μg/ml side) dose-dependently induced a behavioral sensitization to a later challenge with AMPH administered into the nucleus accumbens (1 μg/ml side) whereas the repeated administration of AMPH into the nucleus accumbens (0, 1, 3, 10 μg/ml side)/did not produce such effect. To further specify the mechanism by which these doses were implanted in the VTA, we studied the respective involvement of VTA D1-D2 and -D3 receptors in the behavioral sensitization produced by intra-VTA administration of AMPH. In Experiment 1, 500 μg of DA receptors or DA receptors of the VTA were injected in the VTA following immediately after intra-VTA administration of AMPH (5 μg/ml side) or saline (saline). In Experiment 2, the same protocol was repeated except that AMPH was injected in the DA-D2 antagonist (salam and basal DA). In Experiment 3, the same protocol was repeated except that AMPH was injected in the DA-D2 antagonist (salam and basal DA). Supported by grant DA7221 and by the National Institute of Alcohol Abuse and Alcoholism (NIAAA). All the rats were divided into the following groups: Controls: (1) VTA AMPH pretreatment group, (2) VTA AMPH pretreatment group, and (3) VTA AMPH pretreatment group. The results demonstrated that: 1) VTA AMPH pretreatment group, 2) VTA AMPH pretreatment group, and 3) VTA AMPH pretreatment group. (Supported by grant DAO7722.

Methamphetamine (METH) is one of the drugs of abuse known to cause neurotoxicity in the adult rodent and nonhuman primate. Little attention has been focused on the evaluation of pre- or perinatal exposure to METH on the developing brain. The present study was to evaluate whether pre- or perinatal exposure to METH produces changes in monoamine levels in maternal or developing pup brain. Pregnant CD rats were injected sc with either 0.2, 2.5 or 5 mg/kg METH daily on gestational days 6 through postnatal day (PND) 21. Pups were sacrificed on PNDs 1, 7, 14 or 21 and dams were sacrificed 24 hours after the last dose (PND 22). Monoamine levels were measured by HPLC/EC. Pre- and perinatal exposure to METH had little effect on monoamine levels in different brain regions at PNDs 1, 7, 14 and 21. However, in maternal brain, dopamine and serotonin levels were significantly decreased in the caudate nucleus. It is concluded that pre- and perinatal exposure to METH at these doses produces significant alterations in the maternal but not the developing rat pup brain monoamine levels.


This experiment assessed the ability of (-)-SHT: antagonists to reverse amphetamine-induced facilitation of brain stimulation reward. It has been shown previously that SHT: antagonists alone do not reverse amphetamine-induced facilitation of self-stimulation in the VTA. We speculated that the mixed antagonists would have a greater effect than more specific antagonists. Risperidone (1.8 mg/kg) and MDL 28,133 (1.5, 3, 5, 6, & 7.5 mg/kg) were injected 15 min prior to amphetamine. Both doses of Risperidone and the two highest doses of MDL 28,133 significantly decreased the effects of amphetamine. However, the ability of both drugs to reverse amphetamine's effects could be predicted from the elevation in thresholds produced by the antagonists alone. Therefore, it seems likely that the ability of the mixed antagonists to reverse amphetamine's effects is due to their D2 component.

This project was supported by NIDA grant # DA 04483 and a grant from Marion Merrell Dow. Acknowledgements to Marion Merrell Dow for supplying MDL 28,133 and Janssen Research Foundation for supplying Risperidone.


Substituted amphetamines such as 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA) and fenfluramine (FEN) are agents that affect serotonergic neurotransmission. In order to explore the effects of these agents on serotonergic neurotransmission we have used two different approaches: 1) to examine the effect of these agents on the release of serotonin (SHT) from primary cultures of raphe neurons with a combination of serotonergic neurotransmission. We have used two different approaches: 1) to examine the effect of these agents on the release of SHT from primary cultures of raphe neurons with a combination of serotonergic neurotransmission. We have used two different approaches: 1) to examine the effect of these agents on the release of SHT from primary cultures of raphe neurons with a combination of serotonergic neurotransmission. We have used two different approaches: 1) to examine the effect of these agents on the release of SHT from primary cultures of raphe neurons with a combination of serotonergic neurotransmission. We have used two different approaches: 1) to examine the effect of these agents on the release of SHT from primary cultures of raphe neurons with a combination of serotonergic neurotransmission. We have used two different approaches: 1) to examine the effect of these agents on the release of SHT from primary cultures of raphe neurons with a combination of serotonergic neurotransmission. We have used two different approaches: 1) to examine the effect of these agents on the release of SHT from primary cultures of raphe neurons with a combination of serotonergic neurotransmission. We have used two different approaches: 1) to examine the effect of these agents on the release of SHT from primary cultures of raphe neurons with a combination of serotonergic neurotransmission.

In the first approach, we have used primary cultures of rat raphe neurons maintained in vitro for 7-14 days. In these cultures, we have added these agents (100uM) at various time points. We have found that these agents induce a release of SHT from these cultures. This release is blocked by the specific serotonin antagonist ICS 205-930. In the second approach, we have used primary cultures of rat hippocampal neurons maintained in vitro for 7-14 days. In these cultures, we have added these agents (100uM) at various time points. We have found that these agents induce a release of SHT from these cultures. This release is blocked by the specific serotonin antagonist ICS 205-930.

In conclusion, these results suggest that MDMA, MDA and fenfluramine may induce a release of SHT from these cultures. This release is blocked by the specific serotonin antagonist ICS 205-930. In the second approach, we have used primary cultures of rat hippocampal neurons maintained in vitro for 7-14 days. In these cultures, we have added these agents (100uM) at various time points. We have found that these agents induce a release of SHT from these cultures. This release is blocked by the specific serotonin antagonist ICS 205-930.


Previous studies indicated that the medial septal/ diagonal band region (MS) is a site at which the LC-noradrenergic system, via actions at noradrenergic a-receptors, acts to influence forebrain EEG. Many of these studies, while stimulating or recording in the septum, have failed to isolate the specific effects of a-agonists. The present study was designed to investigate the effects of a-agonists on EEG in the septum, while stimulating or recording in the thalamus. The medial septal area (MS) was isolated in the rat brain by in vitro slices and was maintained in a temperature-controlled incubator. The septal area was stimulated with a square-wave current through a tungsten electrode. The EEG was recorded from the thalamus using an electrode inserted into the anterior thalamus. The septal area was stimulated with a square-wave current through a tungsten electrode. The EEG was recorded from the thalamus using an electrode inserted into the anterior thalamus.

The effects of a-agonists on EEG in the septum, while stimulating or recording in the thalamus. The medial septal area (MS) was isolated in the rat brain by in vitro slices and was maintained in a temperature-controlled incubator. The septal area was stimulated with a square-wave current through a tungsten electrode. The EEG was recorded from the thalamus using an electrode inserted into the anterior thalamus.
The Delta-Opioid Receptor Antagonist Naltrendione Attenuates Amphetamine-Induced Increases in Extracellular Dopamine In the Striatum of Rats, C.A. Schalb*, J.B. Justice, Jr.*, and S.G. Hoffman. Departments of Pharmacology and Chemistry, Emory University, Atlanta, Georgia, 30322.

The opioid receptor antagonist naltrendione has been shown to attenuate both the behavioral and neurochemical effects of amphetamine in rats. Furthermore, amphetamine-induced increase in extracellular dopamine activity is attenuated by intracerebroventricularly administered naltrendione (NTI), a selective delta-opioid receptor antagonist. This suggests a role of central delta-opioid receptors in the regulation of the behavioral effects of amphetamine. Therefore, this research was designed to look at the effects of NTI on the amphetamine-induced increase in extracellular dopamine (DA) in the brain of rats.

Microdialysis performed in the right lateral striatum of rats that were pretreated with an intracerebral (IC) injection of 3, 10, or 30 μg NTI or vehicle. Pretreatment injections were followed 15 min later by cumulative doses of cocaine (0.0, 0.1, 0.4, 1.6, 6.4 μg/kg) at 30 min intervals. The microdialysis probes were perfused at a flow rate of 0.6 μl/min and dialysate samples were collected every 10 min from either the nucleus accumbens (NACC) or striatum (ST).

Amphetamine dose-dependently increased extracellular DA in both the NACC and STR, as reported previously. NTI, 3, 10, and 30 μg IC, significantly reduced the DA response to amphetamine in the STR. In contrast, 30 μg of NTI did not modify the DA response to amphetamine in the NACC. These findings suggest that delta-opioid receptors play an important role in mediating the amphetamine-induced increase in extracellular DA in the STR, but not in the NACC.

(Supported by NIDA Grant DA00451, DA07532, K02 DA00179, K05 DA00028, and NIMH Grant R11-116117.)

**DRUGS OF ABUSE: COCAINE—DOPAMINE**


Post-mortem studies of cocaine overdose victims have shown elevations in striatal dopamine (DA) transporters relative to healthy control brains. We examined whether acutely abstinent (< 72 h) cocaine addicts have increased levels of DA transporters compared to drug-naive controls, and whether post-mortem increases could persist with sustained (2-4 weeks) drug abstinence. Cocaine dependent and healthy control subjects (n = 8 each; ages 35.0 ± 10.1 vs. 33.8 ± 3.9, p = NS) were injected with 10 μCi [123I]β-CIT and imaged from 24-30 h post-injection under sustained equilibrium conditions. The ratio of specific to nonspecific brain uptake (i.e., (pm Marty-scipoticial/), a measure proportional to the binding potential (BP) for each compound, was used for all comparisons. Preliminary results suggest a statistical trend (p = 0.047, two-tailed) toward elevations in striatal DA transporters in acutely abstinent cocaine subjects in comparison to healthy controls (10.2 ± 2.7 vs. 8.2 ± 1.6; mean ± SD). Consistent with apparent increases (0.25%) in [123I]β-CIT binding relative to controls, were within subject reductions (17 ± 1.8%; range, 7.8 - 33.3%) in cocaine addicts (n=5) after sustained (2-4 weeks) as compared to acute (< 72 h) drug abstinence (12.2 ± 2.4 vs. 10.1 ± 1.7; p = 0.003). These results are consistent with preclinical studies which suggest an upregulation of DA transporters in response to chronic cocaine administration, changes which appear plastic and may "normalize" in the continued absence of the cocaine.

**466.3**


A novel series of benztpine analogs of cocaine were synthesized and their affinities for monoamine transporters were determined using [3H]WIN 35,428 to label the dopamine (DA) transporter and [3H]tiboloprop to label the serotonin (5HT) transporter. In contrast to the ND forms of cocaine and cocaine congeners (e.g. WIN 35,428), diaflupropone (+4'-carbomethoxy-3'carboxyfluoro phenylethylamine) and congeners displayed reverse stereoselective effects. The S-form was 200 times more potent (IC50 = 10.9 ± 1.2 nM) than the R-form, which was highly selective for the DA/BHT transporter (324-fold) and was more potent than the 4-dihydro analog. Unlike the phenylethylamine series, other substrates on the aromatic ring tended to reduce the affinity for the DA transporter. This series of compound's challenges recent views on the binding domain of cocaine/congeners substrates and offers unique opportunities for probing the binding domain of the dopamine transporter. DA06303, RR00168, Boston Life Sci., Inc.*; reg.Tm.

**663.4**

INVOLVEMENT OF AUTOREGULATION IN THE EFFECT OF COCAINE (COC) ON MONOMINES MEASURED BY DIALYSIS IN THE RAT VENTRAL SEGMENTAL AREA (VTA). M.E.A. Reith* and N.-H. Chen, Dept. of Basic Sciences, University of Illinois College of Medicine, Peoria, IL 61655.

Dopamine (DA) cell bodies in the VTA have been implicated in the rewarding and stimulatory activity of cocaine. The VTA is innervated by serotonin (5-HT) and norepinephrine (NE)-containing afferents from the raphe nucleus and locus coeruleus, in addition to reciprocal efferent connections. In the present study, DA, 5-HT, and NE were measured simultaneously in dialysates from the VTA of freely moving rats, and the effect of COC was measured in the presence or absence of monoamine autoreceptor antagonists in the perfusate. Sulpiride (25 μM), 10 μM) increased DA output induced by local (30 μM) or systemic (20 mg/kg i.p.) COC, methiothepin (5-HT; 5-HT; 20 μM) promoted local COC-induced 5-HT output, and idazoxan (100 μM) enhanced COC-induced NE output. This is consonant with activation of autoreceptors in the VTA counteracting the effect of local COC; possibly, modulation by somatodendritic 5-HT and NE autoreceptors is more important following systemic COC administration. Sulpiride promoted COC-induced NE output without modifying basal 5-HT/NE output, whereas methiothepin and idazoxan increased the basal output of all three amines without modifying COC-induced output of DANE or DAVs-HT, implying complex monoamine interactions. While methiothepin or idazoxan depressed COC (20 mg/kg)-induced motor activity. The analysis of behavioral/behavioural relationships revealed a positive correlation between dopamine DA output and motor activity in the sulphiride/COC and methiothepin/COC groups, consonant with VTA DA reflecting DA cell firing, and a negative correlation was revealed between dopamine NE output and motor activity in the COC alone and idazoxan/COC groups, consonant with previous DA/NE balance theories.

(Supported by NIDA 03025.)
664.5 FUNCTIONAL AND PHARMACOLOGICAL MODULATION OF SINGLE-UNIT ACTIVITY IN THE VENTRAL TEGMENTAL AREA AND RELATED REGIONS IN THE AWAKE, BEHAVING RAT. Kosoud, A.E., and Chapin, J.K., Hahnemann University, Philadelphia, PA, 9102 USA.

The ventral tegmental area (VTA) is a central element in a system that mediates the reinforcing properties of natural stimuli (such as food), brain stimulation and drug abuse. In the present study, 464 terminal electrodes in the VTA were chronically implanted in the VTA and target regions, including nucleus accumbens (NA) ventral pallidum (VP), mediodorsal thalamus (MD) and prefrontal cortex (PFC) of male Wistar rats. Following surgery from, simultaneous recordings from single neurons and unit clusters were obtained in unrestrained rats using intraventricular and behavioral studies. A substantial number of neurons in the VTA and target regions displayed no change in firing rate relative to behavior, or were unaffected by the peripheral administration of cocaine, dopamine, noradrenaline (NA), and drugs. However, in doses that have been shown to modulate firing rates in anesthetized rats. This suggests that in the awake rat, functional circuits may be more resistant to disruption by drugs. VTA neurons displaying the electrophysiological characteristics of dopaminergic neurons were relatively active in rats at rest, and reduced their firing rates during movement. These neurons were inhibited by amphetamine, and this inhibition coincided with the appearance of behavioral stereotipes (sniffing and head-waving). This pattern was duplicated in neurons in MD thalamicus, suggesting that functional patterns of activation are reflected in multiple sites throughout a neural circuit. A second class of VTA neurons, apparently non-dopaminergic, showed patterns of activity that were exactly the reverse of the VTA DA neurons: i.e., their firing rate was decreed in rats rest, increased during movement, and excited by peripheral administration of amphetamine. Haloperidol reversed both the behavioral and electrophysiological effects of amphetamine. Supported by NIDA DA 08349 and Hahnemann University R01 90414.

664.6 ELEVATIONS AND PHASE FLUCTUATIONS IN NUCLEUS ACCUMBENS DOPAMINE DURING IV COCAINE SELF-ADMINISTRATION. R.A. Wise,* P. Newton, K. Leeb, B. Burnette, P. Cocock, and I. Justice. 2 Cr Streh Bevah Neurobiol Concordia Univ., Montreal, PQ, CANADA H3G 1M8 and Dept Chem, Emory University, Atlanta, GA 30322.

Fluctuations in extracellular nucleus accumbens dopamine and DOPAC levels were monitored in 1-microdialysis samples from rats engaged in intravenous cocaine self-administration. DOPAC levels were elevated to 200-600% of baseline during cocaine self-administration, fluctuating phasically between responses. Each injection caused a phasic, short-lasting increase in dopamine levels, with a decrease prior to the next lever press. This pattern was seen regardless of whether the dose per injection was fixed or varied unpredictably within the sessions. The magnitude of the phasic fluctuations was on the order of 20% of the magnitude of the sustained within-session elevations; dopamine levels never fell close to baseline levels before the animal responded and received another injection. Fluctuations of the same magnitude and with the same time-course were seen when cocaine was injected independent of the behavior of the animals. DOPAC levels increased in the first minutes and decreased with a decrease prior to the next lever press, with no significant recovery between injections. These data are consistent with the possibility that falling dose levels triggers differential responses in the intravenous cocaine self-administration paradigm, but they offer no support for the hypothesis that extracellular dopamine is depleted at the time of response initiation within a self-administration session.

664.7 ACUTE COCAINE ALTERS DOPAMINE SYNTHESIS IN THE NUCLEUS ACCUMBENS OF RATS PRETREATED WITH SALINE, BUT NOT COCAINE OR CPP. D. Johnson* P. Buckley, M. Koza, and D. Mokler, University of New England, College of Osteopathic Medicine, Biddeford, Me, 04005

A paradigm to develop rapid sensitization to the locomotor-activating effects of cocaine and the role of the competitive NMDA receptor antagonists in rapid sensitization was tested. All rats receiving acute cocaine injections (10 mg/kg) showed higher activity counts (P < 0.01) compared to saline-injected controls. No differences were seen among rats receiving acute cocaine which were pretreated once daily for two days with either cocaine or the competitive NMDA receptor antagonist CPP (2 mg/kg). Neurochemical analysis of norepinephrine (NE), dopamine (DA), DOPAC, 5-HIAA, and 5-HIAA in brain homogenates showed decreased (P < 0.05) levels of DA and HVA in the nucleus accumbens (NA) of cocaine injected rats pretreated with saline, compared to saline injected rats pretreated with saline. Neurechemistry in rats injected with cocaine and pretreated with cocaine, CPP, or both was not different from controls in any brain region examined. These results suggest that this paradigm is inadequate to produce locomotor sensitization to cocaine. However, acute cocaine in this model does produce changes in DA synthesis in the NA of saline-pretreated rats, but prior exposure to cocaine, CPP, or both negates this effect. Further behavioral paradigms are being tested, using microdialysis to measure neurocorrelates.

664.8 PHASIC FIRING PATTERNS OF ACCUMBENS NEURONS ARE RELATED TO CONDITIONED STIMULI-associated WITH DRUG REINFORCEMENT DURING COCAINE SELF-ADMINISTRATION IN RATS. R.M. Carrell, V. C. King, and D. S. Deiborder. Center for the Neurobiological Investigation of Drug Abuse, Dept. of Phys./Pharm., Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27157.

We have previously reported that a subset of nucleus accumbens (NA) neurons exhibit phasic changes in firing rate during cocaine self-administration and water reinforcement in rats (Brain Res 626-14-22,1995; Neurosci Abs 19-1857,1993). Of 244 NA neurons recorded in 14 self-administering rats, 24% (58 cells) exhibited a distinctly different neuronal firing patterns time-locked to the drug reinforced response (0.33 mg cocaine/inf, 5.8 sec) in which drug infusion was paired with a tone-light CS complex (20 sec). The purpose of the present study was to investigate the role of the CS in the phasing patterns of NA cells. NA phasic activity could be triggered by the CS alone since similar patterned discharges occurred independent of drug delivery. However, although the CS was present on all trials, phasic activity time-locked to its occurrence did not emerge until after self self-administration responding. This onset could be prolonged further within the session by: 1) decreasing the dose of cocaine (0.16, 0.08 mg/inf) and; 2) pretreatment with the dopamine (DA) antagonist SCH23390 (10 mg/kg). This indicates that a crucial level of dopamine in the NA must be achieved before the CS can elicit NA phasic activity. In addition, the timecourse of CS related NA firing to cyclic dopamine levels in the NA during the session suggests that phasic activity triggered by the CS does not reflect changes in dopamine levels. [Supported by NIDA grants DA0s355 to RMC and DA06634. DA0619 to SAD.]


Time-dependent changes in mesolimbic dopamine (DA) function are believed to play a role in both behavioral sensitization and drug craving experienced during withdrawal from chronic cocaine administration. The present study utilized intravenous (i.v.) cocaine self-administration coupled with intracranial microdialysis in rats to investigate time dependent changes during withdrawal from chronic cocaine exposure. Rats were allowed contingent access to cocaine at 1 and 7 days of withdrawal while the cocaine-pretreated (MDP) and saline-pretreated (MDN) groups were measured from the nucleus accumbens (MDA). A second group of animals received yoked, noncontingent cocaine for 2 weeks and were then administered noncontingent cocaine on days 1 and 7 of withdrawal. In addition, a third group of animals received 2 weeks of yoked saline following noncontingent cocaine for 2 weeks and were then administered yoked, noncontingent cocaine for 2 weeks and were then administered noncontingent cocaine 7 days after withdrawal. There were no significant differences between groups for the overall cocaine dosage or temporal pattern of infusions on days 1 and 7 of withdrawal. Basal extracellular DA concentrations did not differ between any of the groups on days 1 or 7 of withdrawal. Extracellular DA levels were increased throughout the session on both occasions, however, the increases at day 7 were significantly less than day 1 for both cocaine-pretreated and saline-pretreated groups. This suggests that day 1 did not differ between animals receiving chronic yoked cocaine or saline. These results suggest that tolerance to the DA- Elevations of cocaine is not apparent early in withdrawal, but may be present by later phases of withdrawal. The NA may not be directly related to cocaine craving, since DA levels were attenuated after 7 days of withdrawal while responding for cocaine was unaltered.


Cells in the nucleus accumbens are sensitized to the inhibitory effects of dopamine for several weeks following exposure to noncontiguous injections of cocaine (Henry & White, J. Pharm. Exp. Ther., 1991, 258-882). The present study examined the effects of cocaine exposure on responses of nucleus accumbens cells to dopamine and to methylenedioxyamphetamine (MDMA). Rats pressed a lever in an operant chamber to receive cocaine infusions through cannulae implanted in the jugular vein. The average daily cocaine dose obtained by the cocaine self-administering rats was 31.4 ± 1.2 mg/kg, iv. Following 15 daily sessions of cocaine self-administration, access to cocaine was terminated. Electrophysiological experiments were conducted 1-11 days following termination of cocaine self-administration. DOPAC and dopamine produced dose-dependent inhibition of glutamate-evoked firing in the nucleus accumbens of both the cocaine-pretreated group and a saline-control group of animals. However, dopamine and MDMA had significantly greater inhibitory effects in the cocaine self-administration group than in controls. It is concluded that repeated cocaine exposure at doses that are self-administered sensitizes nucleus accumbens cells to the inhibitory effects of dopamine and of other drugs of abuse that elevate extracellular levels of dopamine in the accumbens. [Supported by DA-08116.]

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664.11

The effects of the administration of psychomotor stimulant drugs in experimental animals depend on a series of factors. It has been previously demonstrated that intravenous (i.v.) but not intraperitoneal administration of cocaine in the rat produces increases in glucose utilization in portions of the mesocorticolimbic system, such as the nucleus accumbens (NACC) and the medial prefrontal cortex. Anatomical and biochemical differences have been reported within the NACC, with regard to the density of dopamine neve terminals and the functional connections. In this study, the 2-[14C]deoxyglucose method was applied to measure the effects of the acute i.v. administration of cocaine or amphetamine on glucose utilization in the shell and core of the NACC in freely moving rats. After catheterization of the femoral vessels, animals were treated with either cocaine (1 mg/kg), amphetamine (0.5 mg/kg) or saline. Computer-assisted image overlay was performed in order to identify the different portions of the NACC in histological sections. Administration of either cocaine or amphetamine produced increases in glucose metabolism in the shell of the NACC, with respect to control animals. These results provide an useful basis for the understanding of the neural substrates of the effects of reinforcing doses of psychomotor stimulant drugs.

664.12
ALTERATIONS IN Dopamine UPTAKE TRANSPORTER DENSITY IN COCAINE SELF-ADMINISTERING RATS APPEAR TRANSIENT. J. H. Graham* S. I. Dwarkin and L. J. Porzio. Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

The in vitro autoradiographic distribution of dopaminergic uptake transporter sites (DAT) was determined, using [3H]mazindol (MAZ) as a ligand, in brain sections from Fisher 344 rats chronically self-administering (SA) cocaine (COC) (0.33 mg/kg/ip) in daily 3 hr sessions. Rats were sacrificed either immediately following a SA session, or after 4 days of withdrawal. Values for DMI insensitive [3H]MAZ binding were compared to those from yoked saline controls similarly sacrificed. Although the distribution of MAZ binding sites in SA rats was decreased in both mesolimbic and nigrostriatal systems, DAT densities returned to levels at or above those found in untreated controls, following 4 days withdrawal. DAT levels in the accumbens (shell and core) and olfactory tubercle of withdrawal rats were elevated above control levels, whereas levels in other areas, e.g. caudate, SNc, rostral pole of accumbens, did not differ from controls. Therefore, adaptations in response to chronic COC SA within both the nigrostriatal and mesocorticolimbic dopaminergic systems may be transient in nature, and only persist as long as COC SA continues. (Supported by NIDA grants P50 DA 06634 and DA 07522).

664.13
POSSIBLE MECHANISMS OF BIPHASIC FLUCTUATIONS IN MESOLIMBIC DOPAMINE ACCOMPANYING COCAINE SELF-INJECTIONS: A VOLUMETRIC STUDY. E. A. Kiyatkin* and E. A. Stein. Dept. of Psychiatry at the University of Wisconsin, Madison, WI 53706.

Cocaine is known to have a complex action on the mesolimbic dopamine (DA) system enhancing, due to reuptake inhibition, accumulation of pre-released DA and inhibiting, due to the depletion of DA cells, impulse-dependent DA release. Rapid biphasic fluctuations in nucleus accumbens DA-dependent signal have been reported in our previous electrochemical studies to accompany individual cocaine self-injections (Kiyatkin et al., 1992). Electrochemical signal gradually increased preceding the lever-press for cocaine, and abruptly (<20 sec) transiently (≤ 2 min) decreased after drug injection. Although inhibiting action of cocaine on reuptake of released DA appears to determine the gradual signal increase preceding self-injections, the mechanisms of post-cocaine signal decrease are unknown. To test the contribution of presynaptic auto receptor activation and local anesthetic action to the post-cocaine signal decreases, electrochemical signal changes were studied in cocaine-experienced rats during self-injections of the direct DA agonist amphetamine (AP; 25 μg/kg) and the local anesthetic procaine (PRO; 0.8-3.6 mg/kg). Signal decreases were found after AP self-injections, but they occurred with a definite latency (90-100 sec) that is not compatible with the immediate signal decreases seen after cocaine. Gradual signal increases seen preceding cocaine self-injections were absent in all cases. Thus, although a presynaptic DA auto receptors may contribute to inhibiting action of cocaine on DA release, this relatively slow and long-term effect seems unlikely to mediate the immediate post-cocaine signal decreases. In contrast, an abrupt (<30 sec) signal decrease found after PRO self-injections implicates cocaine's anesthetic action as a possible contributor to the immediate post-drug signal depression. The combination of local anesthetic and reuptake inhibiting properties may underlie the unique abuse potential of cocaine in contrast to agents with only DA uptake inhibiting properties (mazindol, nonfenstine) or local anesthetic properties (lidocaine, procaine).

664.14
TRANSLATION OF DOPAMINE AND BINDING OF WIN 35,428 MEASURED UNDER IDENTICAL CONDITIONS IN RAT STRIATAL SYMPATHTOPES. C. Xu, L. L. Coffey, S. M. Lasley* and M. E. Reith. Dept. of Basic Sciences, University of Medicine, Phoenix, AZ 85054.

One strategy for searching a for a compound that potently inhibits binding of a cocaine-related radioligand to the dopamine transporter but does not a effect dopamine transp. The conditions commonly used for these assays are not the same, confounding data interpretation. In this study, identical conditions have been adopted for the parallel measurement of the binding of [3H](N) 3,4,5,6 and the translation of [3H]dopamine in crude synaptic preparations from rat stratum: 8 min incubation at 20°C with radioligand and inhibitor in phosphate uptake buffer containing Na+ (400 μmol/L), K+ (30 μmol/L), Ca2+ (1 μmol/L), and naltrexone. Binding IC50 values for a series of different uptake blockers (cocaine, WIN 35,428, benzphetamine, nomifensine, mazindol, methylenediphenolet, BTCP, Lu 19-005, DGR 12909, BGR 12956 and CGS 12906) were calculated. Thus, binding IC50 values for F4.4-fold higher than the uptake IC50 values. For slowly equilibrating inhibitors, uptake IC50's decreased by monitoring [3H]dopamine uptake for 1 min only during the last min of the 8 min presence of inhibitor, and under these conditions the average binding over uptake IC50's increased to 2.3. Kinetic calculations, taking into account both radioligand and inhibitor equilibration kinetics, indicated that the latter comparison between binding and uptake measurements was most relevant, and suggested the involvement of complexities beyond simple competitive inhibition of dopamine transport such as different binding domains for substrate and blocker recognition, or spare receptors for blockers. The present results indicate that binding over uptake IC50 ratios should be interpreted with caution depending on the experimental conditions used to measure these ratios. Supported by NIDA 30925.

664.15

The ventral pallidum (VP) is an output structure of the nuc. accumbens. This anatomy suggests that VP may share functions with nuc. accumbens. It is also known that VP is involved in a number of functions related to drug reward. We investigated whether a DA input to VP, from ventral tegmental area has a role in locomotor activity and reward. We prepared rats with chronically indwelling cannula in VP. Drug injections of 0.5 μl were bilateral. Locomotor activity was tested in 1 hr tests in Digican activity chambers. Amphetamine SO4 (10 μg) and dopamine HCl (25 μg) increased activity. Activity was initially suppressed and subsequently elevated by cocaine HCl (12.5-100 μg), the initially suppressed activity was similar to that induced by procaine (50 μg). Place preference conditioning was conducted using a counterbalanced design pairing drug injection in VP with a distinct chamber for two 30 minute sessions. Amphetamine SO4 (10 μg) increased locomotion. Cocaine HCl (50 μg) also increased time spent on the cocaine-paired side. These data indicate that dopaminergic transmission in VP may be involved in the locomotor and reward effects of psychostimulants, and suggest some effects of intracranially administered cocaine are due to local anesthe.}

664.16

Variability in responsivity to therapeutically addictive drugs is a major problem in the replicability of pharmacological research involving both humans and laboratory animals. Recent data from our laboratory (Antelmin et al., 1992) suggest that one source of variability may be the tendency of physiological systems to change their response to repeated drug treatment from a unidirectional to an oscillatory pattern. In the present study, we investigated the effect of cocaine HCl (20 mg/kg, IP.), 30 min. before sacrifice, increased in vitro amphetamine (AM; 10/m) induced DA efflux from striatal slices, compared to controls. This increase was prevented when COC treatments were given 4 d and 30 min before sacrifice. This oscillatory pattern continued through 6 COC treatments (4 d apart). As each COC treatment was added, it reversed the effects of the preceding one. Most importantly, the direction of COC's effects was completely reversed over the 6 treatments. COC increased DA efflux by 53% when given only once but decreased it by 32% after 6 treatments. In a subset of rats, this oscillatory pattern also was seen for DA-efflux in the nucleus accumbens in response to 4 COC pretreatments. Supported by MH 24114 and DA 07546.

664.17

EFFECTS OF CHRONIC COCAINE ADMINISTRATION ON DOPAMINE TRANSPORTER mRNA LEVELS. S.R. Letchworth, T.A. Hedgecock, L.J. Perone, Dep. of Physiology & Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157 USA.

Repeated administration of cocaine produces sensitization, as seen in increased locomotor behavior. Changes in the density of dopamine transporter protein have been reported in rat striatum after chronic cocaine exposure, but the site of action was not determined. This study was designed to investigate possible changes in dopamine transporter mRNA as a result of chronic cocaine treatment. Male Sprague-Dawley rats were treated with 0, 10, 15, or 25 mg/kg cocaine, ip, once daily for 8 days, and locomotor activity recorded. Two of four cocaine treatment groups were determined autoradiographically using in situ hybridization with an oligonucleotide probe specific for the transporter. The mean density/area was measured in the substantia nigra ventralis and tegmental area. In vitro autoradiography using [3H]-mazindol to measure the density of the dopamine transporter protein will be carried out on tissue from the same animals. Cocaine treatment resulted in decreased levels of dopamine transporter mRNA by approximately 20% in both midbrain regions as compared to control levels. In contrast to the dose-dependent effects of repeated cocaine treatment on locomotor activity, however, the changes in dopamine transporter mRNA were not dose-dependent in either the substantia nigra compacta or ventral tegmental area. The lack of correlation between these two measures suggests that regulation of the dopamine transporter may not be critical for the expression of cocaine-induced sensitization. Supported by NIDA Grant DA07522.

664.18


We have previously reported that rats will self-administer microinjections of MK-801 (a non-competitive NMDA receptor antagonist), nomifensine (a dopamine [DA] reuptake inhibitor), or PCP (a drug with both of these properties) directly into nucleus accumbens septi (NAS). Because other studies have reported that DA reuptake inhibitor cocaine is self-administered into prefrontal cortex (PFC) but not NAS, we explored the possibility that these drugs would also be reinforcing when microinjected into PFC. Rats learned to lever press when given response-contingent microinjections of PCP or MK-801 directly into PFC at the same concentration of each drug that was effective in NAS (12 nanograms 120 nl infusion, respectively). However, when lever-pressing was reinforced with microinjections of NOM into PFC at a concentration that increased responding when given into NAS (1.7 mmol/l), rats of responding were not higher than those of animals that received response-contingent microinjections of vehicle. Higher concentrations of NOM were not tested due to poor solubility. These findings confirm that drug actions in the PFC can be rewarding but, taken together, suggest that it is the actions of PCP at the NMDA receptor rather than PCP-induced blockade of DA reuptake that accounts for its rewarding effects in this brain region. This suggestion—and the fact that NOM was self-administered into PFC—remains to be reconciled with the previous report that cocaine is self-administered into PFC but not NAS.

664.19

BEHAVIORAL EFFECTS OF NOVEL 4- and 4',4'-SUBSTITUTED-3'-DIPHENYL METHYLOXYTROPIANE ANALOGS. L. Katz, S. Jenzewski, A.C. Allen, A.H. Newman, Drug Development Group, Psychobiology Section, NIDA Intramural Research Program, NIH, Baltimore, MD 21224, U.S.A.

Cocaine-like behavioral effects of a series of 4- and 4',4'-substituted-3'-diphenylmethoxy-1H,5H-tropane analogs were assessed. These analogs bear structural features with the cocaine molecule, the dopamine uptake inhibitor, GBR 12909; they bind with high affinity to the dopamine transporter and inhibit dopamine uptake. Cocaine and GBR 12909 produce dose-related increases in locomotor activity of Swiss-Webster mice, whereas, of the diphenylmethoxytropane analogs tested, only the 4',4'-difluoro- substituted compound (AHH 1-055) produced increases in this activity that approached those of the reference drugs. In another study, rats were trained to discriminate saline from cocaine (1 mg/kg, ioi). After cocaine injections, 20 consecutive responses on one of two levers produced food; after saline, responses on the other lever produced food. Once performance was stable, effects of various doses of cocaine, and of the diphenylmethyltropane analogs were assessed during test sessions in which 20 consecutive responses on either lever produced food. Cocaine produced a dose-related increase in responding on the cocaine lever, reaching 100% at 10 mg/kg. Only AHH 1-055 (17 mg/kg) and the 4',4'-dimethoxy analog, AHH 1-057 (5.6 mg/kg), produced effects different from saline. These compounds produced maximum percentages of cocaine responding of 65 and 35, respectively. With AHH 1-057, doses higher than 5.6 mg/kg produced less cocaine responding. With AHH 1-055, doses higher than 17 mg/kg could not be tested because they virtually eliminated behavior. These compounds represent the first tropane analogs that have structural and neurochemical similarities to cocaine, but are behaviorally distinct from all other known compounds that share this profile.

664.20


A series of 4- and 4',4'-substituted diphenylmethoxy-1H,5H-tropane analogs have been prepared as probes for the dopamine transporter. Initial studies, testing the 4'-chloro-3'-diphenylmethoxytropane displaced [3H]WIN 35,428 binding (Kd=30 nm) more potently than cocaine, inhibited uptake by 50%, and did not display a cocaine-like behavioral profile in locomotor activity or drug discrimination studies. Sensitivity to phenyl ring substitution in this series suggested an additional binding domain that might be exploited for the identification of potential cocaine antagonists. In order to further explore the structure activity relationships in this series of compounds, binding to the dopamine transporter, inhibiting dopamine uptake and producing cocaine-like behavioral effects, an extended series of analogs, substituted in the para-position of one or both phenyl rings was prepared. These compounds were evaluated in radiolabeled binding assays for the dopamine transporter ([3H]WIN 35,428), noradrenergic transporter ([3H]desmethylimipramine), and the serotonin transporter (K[3H]paripitram). All of these compounds noncompetitively displaced [3H]WIN 35,428 binding in rat caudate putamen with Kd values ranging from 11-2000 nm. None of the compounds displaced >60% of [3H]desmethylimipramine binding (10 μM) or 46% of [3H]paripitram binding (10 μM). Therefore, the most potent dopamine uptake inhibitors are highly selective for the dopamine transporter. Furthermore, since the parent drug benztpine is a potent muscarnic antagonist, these compounds were also evaluated for binding to muscarnic-m1 receptors ([3H]pirenzepine) and muscarnic-m2 receptors ([3H][AFAI-DX 384]. All of the the analogs displayed moderate to high affinity for the muscarnic-m1 sites (Kd range 1-110 nm) and variable affinities for the muscarnic-m2 sites (Kd range 2-1500 nm). Structure activity relationship trends are evident and distinct for each of the binding sites and suggest that it will be possible to prepare more selective ligands for the future.

664.21

LOCOMOTOR STIMULANT ACTION OF THE DOPAMINE UPTAKE INHIBITORS GBR-12909 AND GBR-12909 CO-TREATED WITH ADDITIVE AND NON-ADDITIVE INTERACTIONS WITH COCAINE. D. Krug, M.J. Forner, M. Stokely, and J. Ebe, Department of Pharmacology, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX 76107.

A study was conducted to compare the locomotor stimulant properties of cocaine with the dopamine uptake inhibitors GBR 12909 and GBR-12909. Horzontal locomotor activity of Swiss Webster mice was recorded for 1 h in a Digiscan apparatus fowing i.p. injection of each drug alone or in combination with 5 to 20 mg/kg cocaine.CONTROL animals, both compounds produced stimulation of locomotor activity with ED50 values of 5-8- and 5-4 mg/kg for GBR-12909 and GBR-12909, respectively. Maximal motor stimulation produced by these drugs was nearly equivalent to that produced by cocaine. Behavioural effects persisted for at least 1 hour following injection. In interaction studies, GBR-12905 produced a drug-related leftward shift in the inverted U-shaped cocaine dose effect curve, whereas, GBR-12909 produced a leftward shift of only the ascending portion. Maximal doses of GBR-12909 failed to influence the response to 20 mg/kg cocaine. These results indicate a non-additive influence of GBR-12909 and cocaine in the modulation of locomotor activity. Supported by U.S.D.H.H.S.-P.H.S. contract NO-DA-2-9305.

664.22

THE RELATIONSHIP OF DOPAMINE CONCENTRATION IN BRAIN TO BEHAVIOR: EVIDENCE FOR THE PREFRONTAL CORTEX AS THE INITIATING SITE FOR THE COCAINE LOCOMOTOR STIMULANT EFFECT. Robert J. Carey, Ernest N. Damaj, and Paul P. De Fusco, Psychology, SUNY Health Science Center and Research and Development Service - 151, VA Medical Center, Syracuse, NY 13210.

The behavioral stimulant effects of cocaine have been linked to cocaine inhibition of dopamine transporter mechanisms in the prefrontal cortex, neostriatum, and limbic areas. In the context of behavioral studies, however, there have been conflicting results as to whether the cocaine concentration was measured in these brain structures. Using a recently developed assay procedure, we examined the effects of IP cocaine (10 mg/kg) administration upon locomotion in the non-naive albino mouse. MAOA deficiency of cocaine in the medial prefrontal cortex, neostriatum and limbic olfactory brain areas. Cocaine concentrations were found to be highest in the cortex followed by the neostriatum and limbic area. Significantly, however, locomotor stimulation effects were reliably correlated only with cocaine concentration in the medial prefrontal cortex. This observation suggested that the medial prefrontal cortex may be the critical initiating site for the mechanisms mediating the cocaine stimulatory effects on locomotor behavior.
664.23

COMPARISON OF COCAINE AND COCAETHYLENE IN THE VERVET MONKEY: PLASMA PHARMACOKINETICS AND EFFECTS ON EXTRACELLULAR DA. C.W. Bradberry*, J.B. Nobletti, R. Ver, P. Jakow, Yale Univ. Sch. Med., Dept. Psychiatry and Laboratory Medicine, and the West Haven Veterans Administration Hospital, West Haven, CT 06516.

Cocaine is a psychoactive molecule resulting from concurrent cocaine and ethanol consumption, during which a transamination converts the methyl ester of cocaine to acetyl-acetone. It is more selective for DA than cocaine and it has a lower affinity for the 5-HT and somatodendritic uptake sites than cocaine. Thus, and the fact that it can be administered to humans in a clinical setting, makes it a unique tool for investigating drug reward mechanisms. Previous work has demonstrated equivalent actions of the two drugs at the dopamine uptake site in the rat both in vivo and in vitro. We have used microdialysis to compare the effect of 1.5 pmol/kg (equivalent to 0.5 mg/kg cocaine)-HCl cocaine and cocaineethylenediamine on extracellular DA in the caudate of the anesthetized vervet monkey following i.v. administration. Samples were collected at 5 min intervals following drug administration. Concurrent plasma samples were collected for pharmacokinetic analysis. Data were fit to an open two compartment model using weighted non-linear fitting (by PC Nonlin ver. 4). Microdialysis results indicated that both drugs caused an identical four-fold increase in extracellular DA following i.v. administration which peaked in the sample collected during the 5-10min post drug period. Pharmacokinetic analysis indicated the terminal elimination half lives were 31 ± 3 for cocaine, and 53 ± 4 for cocaineethylenediamine, p < 0.01. However, because of a larger steady state volume of distribution for cocaineethylenediamine (17.2 ± 2.2 kg vs 2.5 ± 0.4; p < 0.05), the clearances were not different. Supported by DA 08703, DA 0277, DA 04000, The Yale VA Alcoholism Research Center, and a NARSAD Young Investigator Award to CBW.

664.1

COCAINE SELF-ADMINISTRATION PATTERNS MAINLY REFLECT D1 NOT D2 OR D3 RECEPTOR ACTIVATION. J.D. Beluzzi*, S. Koussuth, G. Chinn, N. Khambatki, C. Nguyen, and L. Stein, Dept. of Pharmacology, College of Medicine, University of California, Irvine, CA 92617.

Cocaine self-administration (S-A) is characterized by precisely-spaced response patterns and an "inverted-U" dose-response curve. These characteristics may be duplicated in S-A of D1 agonists (Self & Steins, 1992), but not in D2 agonist. S-A which is characterized by erratically spaced responding and a positively curvilinear dose-response curve (Beluzzi et al. 1993). Here we report that co-administration of a low dose of a D1 agonist converts erratic S-A into cocaine-like and D2-like response rates and patterns. Rats were trained to bar press for i.v. cocaine (750 μg/kg) in daily 3-hr sessions. After baseline rates had stabilized, different doses of PHNO were substituted for cocaine. PHNO alone (Fig. 1) was self-administered erratically at high rates, but co-administration of the D1 agonist SKF 28355 (3 μg/kg) with PHNO (30 μg/kg) induced low-rate responding similar to that seen with the D1 agonist alone (A). When combined with SKF 2536, other D2 (quiprole, N-0923) or D3 (7-OH-DPAT) agonists gave similar results. Our data indicate that cocaine-S-A patterns are reproduced by S-A of D1 but not D2 or D3 agonists, and that D2 or D3 agonists promote when D1 and D2-like agonists are co-administered. (Supported by NIDA grant DA07747.)

665.3


We have previously demonstrated that intra-accumbens injections of the D2-like receptor antagonist sulpiride potently block locomotion induced by systemic administration of cocaine, whereas intra-accumbens injections of the D1-like receptor antagonist SCH-23390 only block locomotion at high doses. In the present study further assessed the involvement of D1-like receptors by examining the effects of systemic SCH-23390 administration on locomotion elicited by intra-accumbens cocaine. Rats received systemic injections of SCH-23390 (0.03, 0.3, 1.0 mg/kg, i.p.) and 10 min later received bilateral injections of either saline or cocaine (100 μg/μl) into the NAC. Locomotion and stereotypic behaviors were then measured for 60 min. This procedure was repeated twice at 6-day intervals. The two highest doses of SCH-23390 decreased cocaine-induced locomotion, as well as baseline locomotion. The magnitude of cocaine-induced locomotion did not change across repeated intra-accumbens administrations. Stereotypic behaviors were not observed following acute or repeated intra-accumbens cocaine administration. In summary, although intra-accumbens SCH-23390 does not potently block locomotion produced by systemic cocaine administration, systemic SCH-23390 potently blocks locomotion produced by intra-accumbens cocaine administration. These findings suggest that D1-like receptors in brain regions other than the NAC are involved in mediating cocaine-induced locomotion. (Supported by DA07747.)

665.2

EFFECT OF COCAINE ON cAMP-ADENYLYL CYCLASE SYSTEM IN RAT STRIATUM. S.K. Sabri, J.M. Davis and J.J. Yadid*, Illinois State Psychiatric Institute, University of Illinois at Chicago, Chicago, IL 60612.

Repeated low doses or a single high dose of cocaine administration produces behavioral sensitization in rats. Also, repeated cocaine administration in rats has been reported to increase forskolin-stimulated adenylyl cyclase (AC) activity in nucleus accumbens. In the present study, we examined the effects of cocaine on the formation of cAMP induced by dopamine and a D1 selective agonist (through D1 receptors). AC was measured by incubating striatal membranes at 37 °C (2 μg protein) and forskolin (acting directly on catalytic unit of AC). Adenylyl cyclase activity in striatal membranes was measured by [32P]-cAMP formation using [32P]-ATP as a substrate. Acute administration of cocaine (10 mg/kg or 40 mg/kg) did not significantly affect cAMP formation 1 hr or 24 hrs post injection, However, when rats were pretreated with a single injection of high dose (40 mg/kg) and then administered a cocaine challenge (10 mg/kg), a significant decrease (about 30%) was observed in the formation of cAMP in the striatum induced by stimulation with D1 agonist. Similarly, a trend towards decreased formation of cAMP was observed in dopamine-sensitive activity, although it did not reach statistical significance. Since under these conditions of cocaine treatment behavioral sensitization is also manifested in rats, present findings raise the possibility that an inhibition of D1-mediated AC activity in the striatum may play a role in cocaine-induced behavioral sensitization.

665.4

EFFECT OF D1 RECEPTOR ANTAGONIST IN THE NUCLEUS ACCUMBENS ON INTRAVENOUS COCAINE SELF-ADMINISTRATION IN THE RAT. A. McGregor and D.S. Roberts*, Life Sciences Research Centre, Carleton University, Ottawa, K1S 5B6, Canada.

These experiments examined whether an antisense oligonucleotide directed against D1 receptor mRNA in the nucleus accumbens of the rat could disrupt intravenous cocaine self-administration behaviour. Rats were trained to self-administer cocaine (1.5 mg/kg i.v.) under a progressive ratio (PR) schedule of reinforcement and then implanted with intracerebral cannulae above the nucleus accumbens. Following re-establishment of stable self-administration behaviour, three injections of the antisense or randomised antisense sequence were administered at 12 hour intervals. Phosphorothioated at all positions, the antisense and randomised antisense sequences were delivered in a saline vehicle (2.5 mmol/l of sodium chloride). A large and significant decrease in break point (BP) was produced following antisense treatment, which took five to six days to return to baseline BP. In contrast, treatment with randomised antisense produced a much smaller decrease in BP, which showed a rapid return to pretreatment BP. Furthermore, acute treatments had no effect on operant responding for food reinforcement under a fixed ratio (FR20) schedule. The results suggest that injection of D1 antisense into the nucleus accumbens caused large reductions in the reinforcing properties of cocaine as measured under the PR self-administration schedule. The lesser effect of the randomised sequence may be due to nonspecific behavioural deficits following such treatment, and indicates that attention must also be given to the nonspecific effects of such oligonucleotide treatments. Supported by NIDA Contract No. NO1DA-3-7002 and MRC of Canada.
665.5 DIFFERENTIAL EFFECTS OF INTRA-ACCUMBENS SULPIRIDE ON COCAINE-INDUCED LOCOMOTION AND CONDITIONED PLACE PREFERENCE. D.A. Baker*, L.E. Oddi†, T.V. Khoury, J.L. Neisewander. Department of Psychology, Arizona State University, Box 871104, Tempe, AZ 85287-1104.

The effects of intra-accumbens administration of sulpiride on the stimulant and reinforcing effects of intravenous (IV) cocaine were investigated using the conditioned place preference (CPP) paradigm. Adult male Sprague-Dawley rats received 7 conditioning trials which consisted of 30-min exposures to two distinctive compartments on consecutive days. On one day of the trial, the rats received bilateral injections of saline or sulpiride (0.05 or 0.2 μg/μl side) into the nucleus accumbens (NAc). Fifteen min later, the rats were placed into a compartment and immediately injected with either saline or cocaine (4.2 mg/kg, IV). On the third day of the conditioning, the rats received sham intra-accumbens injections and 15 min later were placed into the other compartment. punished responding to cocaine was measured on the first and last trial. Cocaine produced an increase in locomotion which was attenuated by both doses of sulpiride. Cocaine also produced an increase in headbobbing, and a decrease in grooming. These behaviors were not affected by either dose of sulpiride. Headbobbing was the only behavior sensitised by repeated administration of cocaine, and this sensitization was blocked by the high dose of sulpiride. Cocaine produced a robust CPP that was not altered by either dose of sulpiride. Conditioned locomotion was evident following saline injections in the drug-paired environment, and acquisition of this response was blocked by the high dose of sulpiride. These findings suggest that D2-like receptors in the NAc mediate cocaine-induced locomotion, but not CPP. This research was supported by DA07730.

665.7 THE EFFECTS OF PARTIAL DOPAMINE D2 AGONISTS ON SELF-ADMINISTRATION OF COCAINE ON A PROGRESSIVE RATIO SCHEDULE IN RATS. R. Ranadiri*, G. Vickers and D.C.B. Roberts. Life Sciences Research Center, Carleton University, Ottawa, Canada K1S 5B6.

There now exists considerable evidence showing that the rewarding effects of cocaine depend on dopamine (DA) neurotransmission. Thus, drugs that interfere with DA function may potentially be beneficial in the treatment of cocaine addiction. Electrophysiological and biochemical studies have demonstrated that the intrinsic efficacy of partial D2 receptor agonists can be rank-ordered from greatest to least as follows: B-HT 920, SND 919, (-)-3-PPP, SDZ 208-912, SDZ MAR 327. We investigated the effects of these partial D2 receptor agonists on the self-administration of cocaine in intravenously self-administer each of four unit doses of cocaine (0.075, 0.15, 0.3 and 0.6 mg/injection) on a progressive ratio (5) schedule and the effects of BHT 920 (0.25 mg/kg), SND 919 (0.1 mg/kg), (-)-3-PPP (1.25 mg/kg), SDZ 208-912 (0.25 mg/kg) and SDZ MAR 327 (0.25 mg/kg) on the breaking points at each unit dose of cocaine were investigated. The results demonstrated that drugs with the least intrinsic activity (SDZ 208-912 and SND 919) did not affect or increased breaking points for cocaine reward. Thus, the effect of these partial agonists on breaking points for cocaine reward is positively correlated with their intrinsic activity at the D2 receptor. The data suggest there may be a point on the continuum where agonists may decrease breaking points for cocaine but not disrupt food-rewarded responding. (Supported by NIDA Contract NO1DA-3-7032).

665.9 DOPAMINE D2 RECEPTOR AGONISTS PARTIALLY REPRODUCE THE DISCRIMINATIVE STIMULUS EFFECTS OF COCAINE. Roger D. Spealman*, Harvard Medical School, New England Regional Primate Research Center, Southborough, MA 01772-9102.

Dopamine agonists with reported selectivity for the D2 subtype of dopamine receptor were studied for their capacity to reproduce the discriminative stimulus effects of cocaine in squirrel monkeys. Monkeys were trained to discriminate cocaine (0.1 mg/kg) from vehicle using a partial reinforcement procedure and subsequently were tested with a range of doses of 7-hydroxy-dipropylaminotetralin (7-OHP-DPAT; 0.001 - 0.1 mg/kg), 2-(N-phenethyl-N-propyl) amino-5-hydroxytryptamine (N-0434; 0.001 - 0.03 mg/kg) and quinolone (0.03 - 0.3 mg/kg) and quinolone (0.01 - 0.3 mg/kg). Each drug engendered dose-related partial substitution for cocaine, reaching average maximums of 60 - 80% cocaine-appropriate responses after the highest doses tested. The degree of cocaine substitution engendered by the D2 agonists was comparable to that reported previously for D1 and D2 agonists. The results are consistent with the view that D2 receptors in the mesolimbic dopamine pathways play significant role in mediating the discriminative stimulus effects of cocaine in monkeys. Supported by DA00499, DA03774 and RRO0168.

665.10 EFFECTS OF DOPAMINE D2 RECEPTOR AGONISTS ON CONDITIONED INDEPENDENT RESPONSE DURING MULTIPLE SCHEDULES OF VARIOUS FOOD AND COCAINE SELF-ADMINISTRATION IN RATS. B. Weissenborn*, U.F. Koob and F. Weiss. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California 92037.

The present series of experiments sought to examine the effects of selective D1 and D2 dopamine receptor agonists (SCH 23390 and raclopride) and a D2 partial agonist (SDZ 208-911) on food and cocaine self-administration in rats while responding for environmental cues associated with the primary reinforcers. Rats were trained on a multiple schedule during which lever-pressing was reinforced with a food pellet and simultaneous presentation of a tone, or with a cocaine infusion and presentation of a stimulus light. Each session was preceded by a 5 min component during which lever presses resulted in presentation of the respective cues only. Non-contingent delivery of food or cocaine prior to this initial component resulted in a significant shift in preference for the tone or light respectively, suggesting that cues had taken on the role of conditioned reinforcers with incentive-motivational value. Systemic administration of SCH 23390 (10 μg/kg) and raclopride (100 and 200 μg/kg) blocked, while SDZ 208-911-025 - 0.5 mg/kg) enhanced the preference shift induced by non-contingent delivery of cocaine. SCH 23390 and raclopride non-selectively suppressed responding following non-contingent food. During the multiple schedule, SCH 23390, raclopride and SDZ 208-911 decreased the inter-reinforcer intervals for cocaine, and at higher doses increased those for food. These data suggest that low doses of D2 agonists and high doses of D2 antagonists decrease the partial reinforcement effect of cocaine but not food under the schedule parameters used here. In addition, selective dopamine antagonists can attenuate cocaine-induced responding for a conditioned reinforcer, implicating both D1 and D2 receptor mechanisms in mediating conditioned stimulus-reward associations. This work was supported by NIDA grant DA 07348, 08467, 04398 (FW and GFK).
665.11
REDUCTION OF THE REINFORCING PROPERTIES OF COCAINE BY A PARTIAL DOPAMINE AGONIST. L. Pulverenti*, D. Smith and G.F. Koob
Dept of Neuropharmacol., Scripps Res. Inst., La Jolla, Ca 92037 and “Mondino-Tor Vergata” Ctr for Exp Neurobiol, Un. of Rome “Tor Vergata”, Rome, Italy

Partial dopamine agonists are a recently characterized novel class of compounds acting at the dopamine receptor site with high affinity and low intrinsic activity. These drugs act as functional antagonists in conditions of high dopamine tone, while they show an agonistic profile in conditions of dopamine depletion (e.g. denervation). Since the reinforcing properties of cocaine seem to depend upon activation of forebrain dopamine neurotransmission, the aim of the present study was to evaluate the effects of acute pretreatment with SDZ 208-911, an aminoergoline with partial dopamine agonistic activity, in rats trained to self-administer cocaine IV (0.75 mg/kg/injection). SDZ 208-911 (0.25-1.6 mg/kg IP) dose-dependently reduced the reinforcing properties of cocaine as shown by a reduction of the inter-reinforcement interval in rats self-administering cocaine IV with limited daily access. The pattern of responding was similar to that of animals self-administering a lower dose of cocaine (0.37 mg/kg/injection) and to that of animals pretreated with a dopamine receptor antagonist. Cocaine abuse in humans is characterized by intake of high amount of drug followed by withdrawal. Since partial dopamine agonists also show an agonistic profile in conditions of low dopamine tone (i.e. cocaine abstinence), the potential use of these compounds for effective pharmacological intervention during the various phases of cocaine addiction is a hypothesis worth testing.

665.13
SENSITIZATION TO COCAINE SELF-ADMINISTRATION FOLLOWING CHRONIC (Z)-FLUPENTIXOL (FLU), RESULTS IN AN INCREASE IN THE RATE OF COCAINE SELF-ADMINISTRATION. (E.g. Ellenberg et al., Psychopharmacology. 78: 204, 1982). The present study investigated the effects of acute and chronic administration of FLU on the dose-effect curve for cocaine self-administration. Rats were implanted with chronic indwelling jugular catheters. Following implantation, they were allowed to self-administer cocaine (0.25 mg/kg/injection) on a fixed-ratio two (FR2) schedule of reinforcement, 15 reinforcements a day, until baseline responding was stable. Using a multi-dose procedure, a dose-response curve for each rat was then obtained. Rats were then assigned to one of two groups. One group received a 3 hr pretreatment with FLU (0.032, 0.1, or 0.32 mg/kg, i.p.) followed by a multi-dose test. The other group was treated with FLU chronically (0.32 mg/kg/12 hr; s.c.). The chronic regimen lasted for five days, during which rats did not have access to cocaine self-administration. Seventy-two hours after the last chronic injection, cocaine dose-response curves were re-obtained. Acute treatment with FLU produced a dose-dependent shift to the right of the cocaine dose-response curve. In contrast, three days after terminating chronic FLU, a 2-fold shift to the left of the dose-response curve was obtained. The present data suggest that acute dopamine blockade results in a decrease in the reinforcing properties of cocaine as observed by a shift to the right of the cocaine dose-response curve. In contrast, three days after terminating the chronic blockade of dopamine receptors by FLU, there was an increase in the reinforcing properties of cocaine as observed by a shift to the left of the cocaine dose-response curve. Supported by NIDA ROI 4137.

665.14
COCAINE INDUCED LOCOMOTOR HYPERACTIVITY IN THE RAT IS REDUCED BY QUINACRINE. Kevin H. Souta, Aram J. Javath*+, Malcolm S. Reid, S. Paul Berger, UCSF/VAMC, Psychiatry Services 127, 4150 Clement St., San Francisco, CA 94121; * Res, Svc 151-FP, VAMC, 3710 SW Veterans Hosp. Rd., Portland, OR 97201

Quinacrine is a phospholipase A2 (PLA2) inhibitor which is clinically used for the treatment of malaria. As a PLA2 inhibitor one of its main effects is the reduction of arachidonic acid levels. The release of arachidonic acid has been suggested to play a role in dopamine D2 receptor signal transduction mechanisms. In the present study we have investigated whether quinacrine has any effects on the acute locomotor and stereotypic behavioral responses to cocaine in Sprague-Dawley rats. Animals were pretreated with quinacrine (16 mg/kg, i.p.) 15 min prior to cocaine (30 mg/kg, i.p.) administration and locomotor activity and stereotypy were scored every 10 min for 1 hr in an Actimex box. Pretreatment with the cocaine stimulation of locomotor activity by approximately 30%, but had no effect on the stereotypic behavioral response. Quinacrine alone had no effect on locomotion and stereotypy. The ability of quinacrine to modulate caffeine induced locomotor stimulation was also tested, and it was found that quinacrine had no effect on caffeine. These findings suggest that the ability of quinacrine to reduce drug induced stimulation of locomotion is mediated via the dopamine system. Further studies on the behavioral effects of selective D1 and D2 receptor agonists are underway.

665.12
INCREASED SENSITIVITY TO THE LOCOMOTOR DEPRESSANT EFFECTS OF A DOPAMINE ANTAGONIST DURING COCAINE WITHDRAWAL IN THE RAT. B.A. Baldet and G.F.Koob
Dept of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Clinical studies have identified a behavioral syndrome, occurring in stimulant abusers after prolonged cocaine binges, which is characterized by psychomotor depression and anhedonia. This "cocaine withdrawal syndrome" has been hypothesized to contribute to relapse in cocaine users; accordingly, much effort has been dedicated to the development of animal models of cocaine withdrawal in order to elucidate its underlying mechanisms. Recent studies in this laboratory have determined that thresholds for rewarding brain stimulation are elevated following prolonged self-administration sessions in the rat. The post-cocaine threshold elevation was reversible by the dopamine receptor antagonist bromocriptine, suggesting that impaired dopaminergic function may contribute to cocaine withdrawal. In order to determine additional behavioral correlates of cocaine withdrawal, and to further study the role of the dopamine system in cocaine withdrawal, spontaneous locomotor activity was measured in photocell testing cages 4 hr following 12 hr cocaine self-administration sessions. Prior to locomotor activity testing, cocaine-exposed and control animals were injected with saline, or 0.05 mg/kg cis-flupenthixol, a dopamine receptor antagonist. Locomotor activity was found to be significantly depressed in cocaine-exposed animals, but not control animals, treated with cis-flupenthixol. In a separate experiment, doses of cis-flupenthixol as high as 0.4 mg/kg failed to produce significant locomotor depression in drug-naive animals. It is hypothesized that dopamine receptor blockade by a low dose of cis-flupenthixol may interact with cocaine-induced neurochemical changes to produce locomotor depression during cocaine withdrawal. Supported by DA04398 (GFP) and an NSF Predoctoral Fellowship (BAB).

665.15
DOPAMINE RECEPTOR BLOCKADE ALTERS PREPRODYRorphin AND ZIF/268 mRNAs IN RATS THAT “BINGE” ON COCAINE. J.B. DeMeyer*, J.D. Wang, W.T. Bohrer, D.C. Mayer, and J.F. McGuire
Dept. Anat. & Cell Bio., East Carolina University School of Medicine, Greenville, NC 27858-4534

A single injection of cocaine increases expression of preprodynorphin mRNA, whereas repeated cocaine increases dynorphin immunoreactivity (Dyn-ir) and preproendorphin (PPE), but not preproenkephalin (PPE), mRNA in rat striatum. Cocaine’s effects on c-fos and zif/268 mRNAs, or on DA D1 receptor blockade on c-fos, zif/268, and PPD mRNAs after "binging" are unknown. In an effort to answer these questions, thirty adult, male Wistar rats were injected i.p. with SC123390 (0.5 mg/kg) or the D1 receptor antagonist, sulpiride (50 mg/kg) 30 min prior to 3 hourly injections of i.p. saline or 20 mg/kg cocaine for 1 day. One hr after the final injection, the rats were anesthetized and decapitated. Sections were collected through the anterior striatum at the level of the nucleus accumbens, and hybridized with 40-mer oligonucleotides coding for c-fos or zif/268, or 48mers coding for PPE or PFE. Quantitative analysis of autoradiograms revealed that SC123390 completely blocked the cocaine-induced increase in striatal zif/268 and PPD mRNAs. Furthermore, SC123390 alone significantly decreased the basal levels of zif/268 and PPD mRNAs. Sulpiride completely blocked cocaine-induced PPD mRNA and significantly decreased the induction of zif/268 mRNA in the striatum. Sulpiride alone decreased basal levels of PPD mRNA but enhanced the expression of zif/268 mRNA as compared to basal levels in saline-treat rats. C-fos mRNA was undetectable and PPE mRNA remained unchanged by the repeated administration of cocaine. These data demonstrate that: 1) the D1 receptor plays a substantial role in modulating the tonic expression of PPD mRNA in the striatum, a role attributed primarily to the D1 receptor, 2) PPD and zif/268 gene expression is modulated by both D1 and D2 receptors following a cocaine "binge". Supported by DA 01982.
666.1

COMBINED SPECT AND QUANTITATIVE EEG STUDIES IN COCAINE ABUSERS. J.W. Grzynow, L.M. Konopka, T. Miko, E. Barnes, and P. Shiriati. Biological Psychiatry Section and Nuclear Medicine Service, Hines VA Hospital, Hines, IL, 60142.

To examine the relationship between cerebral blood flow as measured by HMPAO-SPECT and brain electrical activity as assessed by quantitative EEG (qEEG) in cocaine abusers, we studied 9 chronic cocaine abusers and compared the results with nine normal controls. HMPAO was injected for SPECT while the subjects were continuously monitored for EEG activity under carefully controlled conditions. Using a novel data-reduction technique, we extracted a single set of group images for comparison with the normal control group. All of the cocaine abusers had abnormal SPECT scans. Using a subtraction topographic maps, cocaine abusers showed areas of hyperperfusion and hypoperfusion. Hyper-perfusion was particularly notable in frontal regions. This hyperperfusion contrasts with other studies in which only hyperperfusion areas were noted. In addition, there appear to be small right-left asymmetries in the perfusion pattern.

Quantitative EEG studies of the cocaine abusers showed increased total alpha power compared to the controls. The increase in total activity came from two sources: first, a widening of the alpha peak indicating a spreading out of the distribution curve of alpha frequencies within the alpha band; and second, a significant penetration of the normally occipital alpha activity into more frontal regions. Combining SPECT and qEEG revealed a strong correlation between increased frontal alpha activity and frontal hyperperfusion as assessed by SPECT.

666.2

REGIONAL BRAIN BLOOD FLOW DURING INDUCED COCAINE CRaving A.R. Childress, D. Mosley, J. Fitzgerald, M. Revicz, J. Legg, and C.P. O'Brien*. Depts. of Psychiatry and Radiology, Univ. of Penn. School of Medicine, Philadelphia, PA 19104.

Human cocaine users can experience profound drug desire when they encounter cues (other drug users, drug-buying or drug-using locations, drug paraphernalia, etc.) which remind them of cocaine, but not when they are in a neutral environment. Cocaine-related cue stimuli can cause an increase in the brain correlates of this desire. Clinically, cue-induced cocaine craving is often accompanied by a number of signs and symptoms similar to the effects of cocaine itself, including generalized arousal, palpitations, light-headedness, ear-ringing, chest tightness, the taste of cocaine in back of the throat, and even mild euphoria. The drug-like nature of these responses suggests that brain structures activated during cocaine craving may be among those activated by cocaine itself, but also that these structures may be particularly limited in cocaine’s pleasurable effects.

We are testing whether limbic regions may be differentially activated during cocaine craving by measuring regional cerebral blood flow (rCBF) with Positron Emission Tomography (PET) and radioactively-labeled water (H2O18) as the flow tracer. Cocaine patients (n=5 thus far) are imaged during exposure to ambient room stimuli (Baseline), and to videos of Neutral (no-drug) and Cocaine-related scenes. Each subject's functional PET images are co-registered with an MRI for anatomical localization of imaged radioactivity. Change in rCBF from Baseline is calculated for both Neutral and Cocaine scenes across selected brain regions. Initial results show the Cocaine video triggered craving, and rCBF during the Cocaine video increased in several limbic regions. Systematic increases in rCBF did not occur in reference areas, e.g., whole brain or hemispheres or in response to the Neutral Video. These preliminary data suggest limited activation may be one component of drug craving.

It has been previously shown that basal corticosterone-secretion facilitates behavioral effects and the rewarding properties of stress stimuli in animals. To further investigate the role of corticosterone in the dopaminergic system, we examined the influence of glucocorticoid treatment on the rewarding properties of cocaine and heroin in rats. We also investigated the effect of glucocorticoid administration on the rate of self-administration of cocaine and heroin by female rats on thebasis of the hypothesis that glucocorticoid treatment could decrease the rate of self-administration of these drugs. These results suggest that glucocorticoid treatment can modify the rewarding properties of these drugs in rats.

Modulation of the discriminative stimulus (DS) effects of cocaine by morphine was investigated in rats trained to discriminate a relatively low (3 mg/kg) or a relatively high (10 mg/kg) dose of cocaine from vehicle. When tested alone, cocaine (0.3 - 18 mg/kg) engendered dose-related increases in cocaine-appropriate responding across both training conditions, with ED₅₀ values being about 3-fold greater under the high-dose training condition than under the low-dose training condition. Morphine (0.3-5.6 mg/kg) did not engender dose-appropriate responding under either training condition. Pretreatment with morphine did, however, enhance the DS effects of cocaine (0.3 - 3.0 mg/kg) under both training conditions, with a dose of 2.5 mg/kg being dose-related to a concomitant reduction in ED₅₀ of 8- to 12-fold. The results are consistent with those previously reported in squirrel monkeys and provide relevant information about the boundary conditions under which morphine-cocaine interactions are observed.

Choline acetyltransferase activity is reduced in rat nucleus accumbens after unlimited access to self-administration of cocaine. J.M. Carroll, S.T. Lac, I.M. DSouza and S.J. Kish. Clarke Institute of Psychiatry, Toronto, Ontario and University of Minnesota, Minneapolis.

Activity of choline acetyltransferase (ChAT), the acetylcholine synthesising enzyme, was measured in discrete areas of rat brain after chronic, unlimited access to self-administration of cocaine. The duration of drug exposure was 6 weeks, during which time the rats self-administered a mean daily dose of approximately 90 mg/kg. Rats were sacrificed either on the last day of cocaine access (n=10) or after three weeks drug withdrawal (n=8). As compared with the controls (n=15), ChAT activity was slightly reduced in striatum (-10%, P<0.05) and moderately reduced in nucleus accumbens (-26%, P<0.05) on the last day of cocaine access. After three weeks withdrawal from cocaine, ChAT activity was still reduced (striatum -18%, P<0.05; nucleus accumbens -32%, P<0.05) relative to controls. These data suggest that self-administration of cocaine is associated with a long-lasting reduction in ChAT activity. Such a reduction in ChAT activity suggests reduced activity of dopamine receptor cholinergic neurones in the basal forebrain, which could underlie some of the behavioral effects of cocaine. (Supported by NIDA grant DA07182).


Considerable evidence, obtained by studies in man and experimental animals, indicates that NPY plays a role in behavior. For example, NPY appears to be an endogenous anticonflict/anxiolytic agent, whose action depends on activation of NPY-Y1-receptors (Wahlstedt et al., Science 259, 528-31, 1993). We have hypothesized that the reduction of forebrain NPY levels (and reduced NPY-Y1-receptor occupancy) resulting from repeated cocaine treatment is associated with severe anxiety and depression-like states that often follow cocaine withdrawal (Wahlstedt et al., PNAS, 88, 2078-82, 1991).

The NPY mRNA and NPY-Y1-receptor mRNA levels in frontal cortex, peripheral cortex, nucleus accumbens, hippocampus and in the hypothalamus were measured with a solution hybridization assay using 300 bp fragments of respective cDNA. The hybridization was linear to 500 pg for both NPY and NPY-Y1-receptor sense transcripts. After 24-hour treatment with cocaine (10mg/kg bw i.p., twice daily), NPY mRNA levels decreased between 20 and 70% in the assayed brain regions. Cocaine treatment for 72 hours, and up to 7 days, further decreased NPY mRNA levels in the sampled regions. NPY-Y1-receptor mRNA levels, on the other hand, increased by 60% in the sampled regions) after 24 hours and stabilized at 70-185% increase after 72 hours.

Our data thus indicate that treatment with cocaine causes profound alterations in NPY and NPY-Y1-receptor mRNA levels. However, the temporal patterns of these effects are distinct for the two mRNA species. (Supported by DA06805).

U50,488, A KAPPA AGONIST, ATTENUATES COCAINE-INDUCED INCREASES IN EXTRACELLULAR Dopamine IN NUCLEUS ACCUMBENS. J.M. Maisonneuve* and S.D. Glick. Dept. Pharm/Tox, Albany Medical College, Albany, NY 12208 and the Capital District Center for Drug Abuse Research and Treatment, Albany, NY 12208

Recent observations suggest that manipulation of endogenous opioid systems may modify the dopamine-dependent effects of cocaine. The combination of the kappa opioid agonist, U50,488, a selective kappa opioid-receptor agonist, produces a partial enhancement of their reinforcing effects and is commonly referred to as "speed ball" by abusers (Masukawa et al., 1993). In contrast, kappa opioid agonists have been shown to attenuate the discriminative stimulus effects of cocaine (Spealman and Bergman, 1992). Using in vivo microdialysis in awake and freely moving male Sprague Dawley rats, we investigated whether U50,488, a selective kappa opioid-receptor agonist, combined with cocaine produces a differential enhancement of the reinforcing effects of cocaine. Cocaine (20 mg/kg, i.p.) produced a ten-fold increase in extracellular dopamine in the nucleus accumbens. Cocaine (20 mg/kg, i.p.) produced a 50% decrease in the effect of cocaine on DA levels. This attenuation was completely reversed by administration of nor-binaltorphimine (10 mg/kg s.c.), a kappa antagonist, 20 minutes before the agonist challenge. These findings indicate that activation of kappa receptors attenuate cocaine's effects and thus kappa agonists may have a potential role in the pharmacological management of cocaine addiction (supported by DA03817 and by the Aaron Foundation Diamond).
666.19 Glycine/NMDA antagonist, (+)-HA-966, prevents behavioral sensitization to cocaine and subsequent effects on aversive conditioning. Brent A. Morrow, Jane R. Taylor and Robert H. Roth. Dept. of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06520

The noncompetitive NMDA receptor antagonist, MK-801, has been shown to prevent locomotor sensitization to repeated cocaine. However, MK-801 has been shown to cause locomotor sensitization to itself. We tested (+)-HA-966, a 1-hydroxy-3-aminopyridine derivative of the glycine agonist (+)-glycine, on the NMDA receptor complex, on locomotor sensitization to repeated cocaine administration. Vehicle or (+)-HA-966, 15 mg/kg ip, was given 30 min prior to vehicle or cocaine, 15 mg/kg ip, daily for 21 consecutive days. Seven larvae, after several latency periods, were sensitized to cocaine, 15 mg/kg. (+)-HA-966 prevented sensitization to the cocaine challenge and did not cause a sensitization to itself. Three weeks later, rats were subjected to aversive conditioning; 10 randomly presented tones terminated with 0.5 sec, 400 mA footshocks over 30 min. The following day the rats were returned to the chamber, given 10 tones without footshock, and sacrificed after 30 min. The medial prefrontal cortex (mPFC) and nucleus accumbens (NAS) were harvested and assayed by HPLC-EC for serotonin and dopamine along with their metabolites. As expected, the conditioned rats had significantly greater immobility (freezing) during and after the tone, increased focal body and elevated dopamine turnover (DOPAC/DACD) in the mPFC and NAS. Prior exposure to cocaine attenuated the increase in behavioral (i.e. freezing and focal body) and blocked some of the biochemical (NAS dopamine turnover) changes seen with cocaine. (+)-HA-966, the glutamate agonist, prevented these effects. The role of the NMDA receptor with regards to cocaine sensitization and subsequent cross-tolerance to aversive conditioning will be discussed.

Supported in part by MH-10492 & MH-14276

666.21 EFFECTS OF INTRAVENTRICAL COCAINE ADMINISTRATION ON GLUTAMATE-INDUCED EXCITATION AND SPONTANEOUS ACTIVITY IN RAT'S CEREBELLAR PURKINE CELLS. C. A. Jiménez-Rivera*, O. Segura, S. Mato, V. Algranit, J. Dorne and B.D. Waterhouse. Deps. of Pharmacology, UCC, Sch. of Medicine, University of Pau and Rhodes University, Univ. Phil. Pa., 19102.

Cocaine is a well known substance of abuse with central psychostimulants effects and local anesthetic properties. Biochemical studies indicate that cocaine's main action is to increase central synaptic levels of monoamines through blockade of reuptake mechanisms. This study investigated the effects of intravenous (i.v.) cocaine administration on Purkinje cells response to glutamate, a putative neurotransmitter with clear actions in the cerebellum. Excitatory responses of individual neurons to microiontophoretic pulses (10 ms at 10 am perisynaptic; 10^(-4) M) were examined, before, during and after cocaine injections (0.15 and 1 mg/kg, i.v.). At doses of 0.25 mg/kg cocaine induced a significant reduction in spontaneous activity (spikes/sec x 10^4, n = 6) and glutamate-evoked response (37.6 ± 7.2 to 33.9 ± 10.7). Similar effects were observed at doses of 1 mg/kg, however, they were lower in magnitude (5A - 32.6 ± 2.77 to 17.96 ± 4.20, n = 6) and glutamate-evoked response (37.6 ± 7.2 to 33.9 ± 10.7). The major effect was observed within the first 2 min. after cocaine administration. Animals treated with procaine showed no significant changes in either spontaneous or glutamate-evoked excitation. These data suggest that cocaine can modify spontaneous neuronal activity and glutamate-induced excitation in the cerebellum via a mechanism independent of its local anesthetic properties. Studies are underway to elucidate the role of monoamines in this action (Supported by DA 07175 and R01 RR03035 to C.A.J.R.).

666.22 THE ROLE OF NUCLEUS ACCUMBENS DOPAMINE AND EXCITATORY AMINO ACID RECEPTORS IN THE EXPRESSION OF COCAINE-INDUCED BEHAVIORAL SENSITIZATION IN RATS. R.C. Pierce*, M. Adams, B. Born, T. Duffy and P.W. Kalivas. Alcohol and Drug Abuse Program, Washington State University, Pullman, WA 99164 USA.

We monitored the behavioral response to intra-accumbally administered dopamine and excitatory amino acid agonists (amphetamine and AMPA, respectively) following repeated cocaine or saline injections. Our results indicated that both of these drugs dose-dependently enhanced behavioral hyperactivity in cocaine relative to saline-pretrained animals. In separate experiments, using doses 10-fold cocaine or saline injections, we performed in vivo microdialysis in order to determine if changes in accumbal dopamine release might underlie these behavioral effects. The biochemical data revealed that the local administration of both amphetamine and AMPA (through the probe) produced a significant concentration-dependent increase in accumbal dopamine in saline-pretrained animals. Among cocaine pretreated rats, however, there was a potentiation of accumbal dopamine release only following amphetamine administration. Taken together, these results suggest at least two distinct mechanisms that contribute to the expression of cocaine-induced behavioral sensitization: 1) a dopamine-mediated mechanism that is expressed in the putatively sensitive ability of psychostimulants to increase accumbal dopamine and 2) an increase in the functional availability of non-NMDA excitatory amino acid agonists to induce behavioral hyperactivity that is independent from the accumbal dopamine system.

666.23 THE ROLE OF GLUTAMATERGIC ACTIVITY THROUGH NUCLEUS ACCUMBENS AMPA RECEPTORS IN THE EXPRESSION OF BEHAVIORAL SENSITIZATION TO COCAINE. K. B. Bell, P. Duffy* and P. W. Kalivas. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164-6520.

Through two behavioral experiments in the rat, we examined the role of glutamatergic activity through nucleus accumbens (NA) AMPA receptors in the expression of cocaine sensitization. In experiment 1, one group received seven daily IP cocaine treatments, while a control group received an identical treatment schedule with IP saline. For all subjects, open-field locomotor activity was monitored for two hours following first and last treatments to assess the extent of sensitization. On days 20, 21 and 22 of withdrawal from treatment, each subject received bilateral intra-NA microinjection of 0, 0.3, or 1 nmol/side of the glutamate agonist AMPA. All subjects received each microinjection at least once for order effects. Subjects receiving cocaine and displaying sensitization to cocaine (order >20% increase in cocaine-induced activity and cocaine naive) were assessed at 5 min (Po 15 min) cocaine, 15 mg/kg each; showed significantly greater locomotor activity response to 1 nmol/side intra-NA AMPA than either subjects administered saline or cocaine–pretreated subjects which failed to sensitized. In experiment 2, all subjects received the chronic cocaine treatment schedule used in experiment 1. On days 14, 17, 20 and 23 of withdrawal from treatment, each subject received intra-NA microinjection of the non-NMDA receptor antagonist CNQX immediately prior to 15 mg/kg IP cocaine. In subjects which met the sensitization criterion, pre-treatment with 1 nmol/side intra-NA CNQX reduced cocaine-induced hyperactivity to the level seen in response to the first cocaine administration (p<.06). Taken together, the results of these experiments indicate that the expression of behavioral sensitization to cocaine depends, in part, upon sensitization-specific, AMPA receptor-mediated changes in glutamate transmission within the NA.
666.25  
EFFECTS OF THE METABOTROPIC GLUTAMATE RECEPTOR BLOCKER AP-3 ON COCAINE-INDUCED DOPAMINE RELEASE AND LOCOMOTOR BEHAVIOR. P.A. Vincent* and E.L. Gardner, Dept. of Pharmacology, University of Tennessee College of Medicine, Memphis, TN 38163.

Administration of Cocaine (C) has been previously shown to induce dopamine (DA) release in the nucleus accumbens (Nac) using microdialysis. We used this model to identify the neural substrates that modulated C-induced DA release, and we have studied the glutamatergic system because C-induced DA release is one of the substrates from the hippocampus, amygdala and prefrontal cortex, and Glu has been shown to interact with DA function. To study this interaction, we administered the Glu metabolite receptor blocker L-1-2-Amino-3-phosphonopropionic acid (AP-3) through a microdialysis probe placed in the Nac while monitoring DA release. No effect was seen in the Nac 15 min after the administration of 0.1 mM AP-3 (2 µM) or veh was administered to Sprague-Dawley rats treated with C or veh. DA release from animals collected every 10 min for 10 samples. Animals treated with veh and C had an increase in DA release above baseline. Animals receiving AP-3 and plus showed a 160% increase in DA. Animals receiving AP-3 plus C exhibited a 491% increase in DA, similar to C alone. In 15, 0.5, and 1.0 mM AP-3 or veh was infused through bilateral cannulae placed in the Nac 15 min prior to receiving 20 mg/kg C. In locomotor behavior was recorded for the first 2 min of 10 successive 10 min periods. Locomotor activity was not significantly diminished in animals given AP-3 plus C compared to veh plus C. These data suggest that Glu neurons may regulate the same pool of DA that C releases. Further work is needed to determine whether C-induced motor output is modulated by Glu metabotropic receptors. Supported by the Aaron Diamond Foundation.

666.27  

Dopamine, serotonin and norepinephrine transporters are recognized as the main target for cocaine reinforcement and addiction. The digitonin-activated rat brain proteins exhibited cocaine-sensitive [3H]citalopram (CTL), [3H]BFCP and [3H]GR12935 binding. A BFCP analog, reduced citalopram and an amino- cocaine analog were linked to Affigel 10 for affinity purification. They all yielded low µg quantities of proteins that retained cocaine-sensitive binding activity after fast dialysis through ultrafiltration membranes. [3H]CTL gave the largest binding signal of various transporter ligands tested. Its affinity for the serotonin transporter was not altered by solubilization, and it was always recovered in part in the three affinity fractions. The proteins eluted from the anosococaine affinity column exhibited cocaine-sensitive specific binding of [3H]BFCP, [3H]GR12935 and [3H]CTL. SDS-PAGE electrophoresis of the isolated proteins will also be presented.

(Supported in part by NIDA grant # DA06830)

666.28  

Previously we have identified a cocaine analog (RTI-121) that exhibits nanomolar affinity and high selectivity for the dopamine transporter in rat striatum. There was a high correlation (r = 0.0001) between the potencies of drugs to inhibit specific [3H]RTI-121 binding in the striatum (STR) and the potencies of these drugs to inhibit psychotomimetic effects. However, we found the potencies of these compounds to inhibit specific [3H]RTI-121 binding with either [3H]serotonin or [3H]norepinephrine uptake.

When [3H]RTI-121 binding was conducted in the frontal pole (FP) of the rat cerebral cortex several differences were apparent. While [3H]RTI-121 still bound to a high- and low-affinity site, the number of sites labeled by [3H]RTI-121 was approximately 1% that of the striatum. However, when the binding of [3H]RTI-121 observed in the FP was similar to that observed in the STR, several compounds displayed a significantly lower I50 in the FP than in the STR. These included several of the tricyclic antidepressants (imipramine, clomipramine, amitryptiline, nortriptyline, desipramine and desipramine). 

In addition, these sites showed less sensitivity to the effects of 6-OHDA which unlike striatal binding sites were almost completely eliminated by that toxin. These results suggest that [3H]RTI-121 may bind to a yet unidentified site in the cerebral cortex that may recognize cocaine and many antidepressants with high affinity.

667.1  

The losses of clozapine's (Cz) enhanced antipsychotic potency and reduced incidence of motor side effects is unknown. It is conceivable that glutamate, because of its influences on dopamine release and its participation in motor side effects, is a target of Cz's actions. The present work assessed this possibility.

Glutamatergically-mediated field potentials were monitored in striatal slices perfused with Mg2+-free artificial CSF (aCSF). These potentials were suppressed by kynurenic acid or MK-801 and were thus presumed to be mediated, in large part, by NMDA receptors. Cz, added to the aCSF, suppressed these responses in concentrations as low as 10 nM.

Since previous work showed displacement of [3H]MK-801 at much higher concentrations (K50=174µM), Cz has at least two sites of action on the NMDA receptor. The Cz concentration that suppressed responses in striatal slices was similar to that in CSF of patients for treatment of psychosis. Thus, activity at one of these sites suggests that anti-glutamatergic effects may enter into this drug's clinical profile.

667.2  
TYPICAL AND ATYPICAL NEUROLEPTICS DIFFERENTLY AFFECT FOS PROTEIN EXPRESSION IN THE RAT FOREBRAIN. A. Fink-Jensen and P. Kristensen, Pharmaceuticals Division and Biopharmaceuticals Division, Novo Nordisk A/S, Novo Nordisk Park, DK-2780 Måløv, Denmark.

The cellular synthesis of the transcription factor protein Fos is regarded as a biochemical marker of neuronal activity and previous studies suggest that the effect of the atypical neuroleptic clozapine on Fos protein expression in the prefrontal cortex (PFC) may be related to its unique effects on negative symptoms in schizophrenia (Neurosci. (1992) 46:315-328). In order to investigate if the Fos protein expression pattern induced by clozapine applies to other compounds with an atypical profile (preclinical or clinical trials), we investigated the acute effect of the atypical neuroleptics clozapine, risperidone, sertindole and NNC 22-0031 (4-(6-fluoro-1,2-benzisoxa- zol-3-yl)-1-(3-(4-methylenedioxyphenyl)carboxyl)propyl)piperidine) as well as the prototypical neuroleptic haloperidol. The Fos protein expression was assessed by using an immunohistochemical technique. The present study shows that atypical and prototypical neuroleptics can be differentiated on the basis of their ability to induce Fos protein since the ratio between Fos protein expression in the limbic PFC vs. striatal dorsolateral striatum (DLS) was higher for the atypical neuroleptics than observed with the prototypical neuroleptic.
THE ATYPICAL ANTIPTIONSYCOTIC CLOzapine IS A POTENT AGONIST AT HUMAN CHOLINERGIC MUSCARINIC m4 RECEPTORS AND AN ANTAGONIST AT m1, m2, m3, & m5 RECEPTORS. K.M. Dan, D.S. Jones, and A.D. Jones, Pharmaco- 
ie., Central Research Division, Department of Neuroscience, Groton, CT 06340.

Clozapine is an atypical antipsychotic drug that is more efficacious than conventional neuroleptics and does not produce the side effects that previous reports have shown that clozapine binds with high affinity to all 5 muscarinic receptor subtypes (Tur. J. Pharmacol. 1921:205,1991). Clozapine is an antagonist at some of these subtypes, and has been widely assumed to be an antagonist at all of them. Clinically, one of clozapine's notable side effects is hypersalivation. However, since hypersalivation and clozapine-induced dopamine turnover in rats are both inhibited by the muscarinic antagonist atropine, clozapine may have direct cholinomimetic activity (JPET 268:1452, 1994). In the present study the fractional interaction of clozapine with human m1-m5 receptors expressed in CHO cells was characterized. It was discovered that clozapine (EC50 = 75 nM) and full agonist at only the m4 muscarinic receptor subtype, producing concentration-dependent inhibition of forskolin (FSK)-stimulated cAMP accumulation. This effect could be blocked by N-methyl-scopolamine and pirenzepine in vitro. In contrast, clozapine potently antagonized agonist-induced responses at the other four muscarinic receptors. Thus, clozapine was found to block the inhibitory effect of carbachol on FSK-stimulated cAMP accumulation in m2 expressing cells as well as carbachol-induced PI turnover in cells expressing the m1, m3, m4, m5 receptor subtypes. Since the muscarinic receptor in question is found in the rat and the human brain, the present findings suggest that clozapine-induced hypersalivation as well as its effects on striatal dopamine turnover may be due to direct stimulation of m4 muscarinic receptors in these tissues. This agonist action at the m4 receptor may contribute to clozapine's atypical antipsychotic efficacy.

EFFECTS OF SUBCHRONIC CLOzapine AND HALOPERIDOL ON RATS TRAINED IN A FORELIMB TORMOR TASK. S.C. Fowler*, J.A. Stanford and S. Das, University of Mississippi, University, MS 38677.

Rats trained to extend the forelimb through a rectangular hole and exert downward pressure on a force transducer received either the atypical neuroleptic clozapine or the typical neuroleptic haloperidol for 11 consecutive days. Doses were individually titrated daily for each rat in an attempt to achieve a 50% reduction in force on task (TOT). Clozapine treated rats exhibited dramatic tolerance to the drug's suppressive effect on TOT. In contrast, haloperidol treated rats displayed little tolerance. Despite the accentuated tolerance reflected by TOT in the face of escalating doses of clozapine, no tolerance was seen in clozapine's marked slowing of the dominant frequency of oscillations in forelimb force as measured by Fourier analysis of the force-time recordings. Haloperidol did not produce the oscillation slowing. The dissociation between the tendency to respond (TOT) and the oscillator slowing observed for clozapine may reflect effects at different neurotransmitter receptor sites. Supported by MH43429.

LIKE CLOzapine, OLanzapine SLOwS Rats' FORELIMB FREQUENCY OSCILLATIONS IN A PRESS-WHILE-LICKING BEHAVIORAL TASK. J.A. Stanford* and S.C. Fowler, Univ. of Mississippi, University, MS 38677.

Rats were trained to press a force-sensing transducer with one forelimb while licking (water fountain) task while initial peak force (PF), hold force (HF), force frequency oscillations (FREQ), and time-on-task (TOT) were measured. Following extensive experience on the task, subjects were administered olanzapine (OL, 0.5, 1.0, 2.0 mg/kg), a candidate atypical neuroleptic. OL significantly decreased TOT dose-dependently and had a significant slowing effect upon FREQ as quantified by Fourier analysis. These results were compared to clozapine's (CL) similar effects on the same measures previously reported in the task and with haloperidol's (HAL) FREQ acceleration (Fowler, et al., Psychopharmacology, in press). Since typical neuroleptics such as HAL tend to induce motor side effects such as tremor, these findings may reflect the antitremor and atypical properties of OL and CL. Supported by MH43429.


Although clozapine (CLZ) has been shown to be superior to conventional neuroleptics in the treatment of schizophrenia, CLZ's mechanism of action has yet to be completely delineated. A two-lever drug discrimination procedure was employed in order to more precisely characterize the effect of CLZ on the cholinergic system on the discriminative stimulus properties of clozapine. Evidence for both muscarinic and nicotinic effects at CLZ's site has been demonstrated in previous studies. Because many of these cholinergic drugs show high binding affinity for cholinergic receptors, two discrimination groups were trained. One group was trained to discriminate CLZ (5.0 mg/kg, ip.) from vehicle, and another group was trained to discriminate OXO (0.125 mg/kg, ip.) from saline. Male Sprague-Dawley rats (85% B.W.) were used in 15 min. sessions under a FR50 schedule of food reinforcement. After generalization testing with CLZ-trained rats the drug was coadministered with OXO at the 10.0 mg/kg dose (99.3%) in the SCP group (ED50 = 0.690) and peaked at 2.5 mg/kg (97.0%) in the CLZ group (ED50 = 0.751). Complete generalization with OXO was observed in both groups. In the SCP group, the 10.0 mg/kg dose produced peak substitution (ED50 = 3.513). In the CLZ group, the 1.25 mg/kg dose produced maximal DLR (99.7%) (ED50 = 0.114). Methylcholine (MSP) substitution testing and antagonism of the CLZ-cue with oxotremorine (OXO) were also assessed in the CLZ group. MSCP did not substitute for CLZ at any dose. MSCP (1.5 mg/kg) was coadministered (ie., to block the peripheral effects of OXO) with OXO (0.0325, 0.0625, and 0.125 mg/kg), and the highest dose of OXO caused CLZ-DLR to decrease from 96.1% to 67.6%. Results from this study support the notion that the clozapine discriminative stimulus properties in rats are mediated by antagonism at muscarinic receptors.

ASSESSMENT OF CLOzapine's SUBCHRONIC DOSE EFFECTS ON FORCE, DURATION, AND RHYTHM OF LICKING IN RATS. Shyamal Das* and S.C. Fowler, University of Miss., 1921:205,1991). Three doses (1.5, 3.0, 4.5 mg/kg, ip, 45 min) of the atypical antipsychotic clozapine were studied in a subchronic dosing paradigm (at least 7 consecutive days at each dose with 4 or more vehicle only days separate dosing periods) in order to evaluate potential tolerance or sensitization effects in rats. Thirsty rats (n=20) licked water from a force-sensing disk while force-time waveform licking were recorded for a 2-min session. Lick rhythm was quantitated by applying Fourier methods to the force-time waveform records. Behavioral measures were peak force and duration of individual licks and number of licks. 3.0 mg/kg produced a 4.5 mg/kg, modest, but significant, tolerance effects emerged. 1.5 mg/kg produced sensitization effects, and the lick rhythm was the variable most affected by the drug as measured by omega squared. Clozapine's powerful effects on lick rhythm are unlike those of haloperidol (Fowler & Das, PBR, in press) and may reflect effects at serotonin receptors on the hypocoglossal nucleus. Supported by MH43429.

SELF DESTRUCTIVE BEHAVIOR AND CLOzapine DISCONTINUATION. F.G. Moeller*, S.W. Chen*J.0el, Steinberg, M.D.2,3, M. Fulton, 2,3, P. Petty, W.G. Ripper, S.D. Garver, 3, 1 University of Texas Houston Health Science Center 1300 Moursund, Houston TX 77030, 2 Dallas VAMC 3. Univ. of Texas Southwestern Med. School at Dallas. The atypical antipsychotic clozapine has been effective as well as a dopamine antagonist. There have been reports that clozapine induces depressive symptoms in some patients and it has been speculated that this increase is due to the serotonin antagonistic effects of clozapine. In order to determine if patients with pre-existing suicidal or self mutilative ideation or behavior were more likely to have a poor outcome with clozapine than patients without this behavior, logistic regression was performed on data obtained from 729 patients treated with clozapine in the VA hospital system. Variables analyzed in the regression included age, sex, race, prior history of treatment failure, presence of suicidal behavior in the past month or ever in the past, and presence of self mutilative behavior in the past month or ever in the past. Of the 729 patients in this study 32 (4.3%) had suicidal ideation or behavior in the month prior to starting clozapine, 337 (46.2%) had suicidal ideation or behavior ever in the past, 32 (4.4%) had self mutilation in the past month, 337 (17.8%) had self mutilation ever in the past. There was an significant effect of suicidality or self mutilatory behavior on clozapine discontinuation in the logistic regression model. There was no significant correlation with other variables which have been reported previously. Our study does not support suicidality or self mutilatory behavior as increased risk factors for poor outcome with clozapine.
667.1.

**SELECTIVE ACTIONS OF THE NOVEL ATYPICAL ANTI精神病IC DRUG AMPERODIZE IN THE LIMBIC FOREBRAIN.**


Amperodize (APZ) exhibits a multireceptor profile with high affinity for 5-HT, receptor, relatively low affinity for dopamine (DA) receptors, and a rather marked limbic selectivity of action. Recently, in vivo microdialysis studies have revealed that APZ induces a more pronounced increase in DA concentrations in the medial prefrontal cortex (MPC) than in the nucleus accumbens (NAc) or striatum, antagonizes the DA response to amphetamine, but not with classical antipsychotics. This effect of APZ may be due to its selective 5-HT and DA-D2 receptor antagonistic properties. By employing in vivo voltammetry and Fos immunohistochemistry, we examined the effects of APZ on DA concentrations in two subsets of the NAC, the shell and core, that are associated with limbic and striatal functions, respectively, and on c-fos expression in the limbic forebrain and the nucleus accumbens. APZ (1.0 and 2.0 mg/kg), iv, almost exclusively increased DA concentrations in the NAC-shell compared to the core. Similar results were obtained with ritanserin but not with raclopride, which instead increased c-fos expression in both parts. The number of Fos-positive neurons was significantly increased by APZ (5.0 and 10 mg/kg, sc) in the MPC and the lateral septum but not in the striatum of the NAC. Thus, APZ shows a somewhat clozapine-like profile as regards also its effects on c-fos expression in the forebrain. These results emphasize amperodize's limbic selectivity of action, the importance of 5-HT receptor antagonism for such an effect, and strengthen the hypothesis that the therapeutic profile of atypical antipsychotics may be related to their distinctive effects in the mesolimbicocortical dopaminergic system.

667.13.

**DIFFERENTIAL EFFECTS OF ANTISPISYCHIC DRUGS ON EXTRACELLULAR GABA LEVELS IN THE VENTRAL PALLIDUM AND GLOBUS PALLIDUS OF RATS.**

M.A. Chapman* and R.E. See, Department of Psychology, Washington State University, Pullman, WA 99164-4421.

Antipsychotic drugs (APDs) are believed to treat schizophrenia through their effects on mesolimbic dopamine (DA) pathways and produce motor side effects via effects on nigrostriatal DA pathways. In order to these APD-induced changes to affect behavior, neurotransmitter levels must be altered in the output nuclei of these two systems. To test this hypothesis, female, Sprague-Dawley rats were given a subcutaneous injection of haloperidol (1.0 mg/kg), clozapine (30.0 mg/kg), or metoclopramide (10.0 mg/kg). Intracerebral microdialysis was used to assess GABA levels simultaneously in the ventral pallidum and globus pallidus, regions which receive major GABAergic projections from the mesolimbic and nigrostriatal dopaminergic systems. The ventral pallidum, GABA, was analyzed using o-phthalaldehyde derivatization followed by high-performance liquid chromatography with electrochemical detection. Both haloperidol and metoclopramide increased extracellular GABA levels in the globus pallidus, while clozapine decreased GABA in this region. Clozapine also decreased extracellular GABA levels in the ventral pallidum, whereas haloperidol and metoclopramide had no effect. These results suggest that increased extracellular GABA levels in the globus pallidus may be related to the motor side effects of these drugs, while decreased GABA levels in the ventral pallidum may be related to the unique clinical profile of clozapine.

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The predictive validity of catalysis as a rodent model for detecting EPS of antipsychotic drugs was re-examined when this novel antipsychotic saxopenex produced little catalysis in rodents while producing significant EPS in schizophrenic patients. Because catalysis is viewed as an important model for predicting EPS, we decided to re-evaluate the effects of saxopenex. Saxopenex, haloperidol, clozapine, raclopride, risperidone and ORG 2522 were examined in this test for catalysis (Grip Tests) in male Sprague-Dawley rats. The ability to antagonize amphetamine-induced hypermotility was also examined since this measure is believed to predict clinical efficacy. With the exception of dopamine, all drugs produced dose-dependent catalysis in both tests. For each drug, the MEDs for producing catalysis were greater than those necessary for antagonizing amphetamine-induced activity. Clozapine demonstrated the widest separation of MEDs in the catalysis and activity models while haloperidol showed the narrowest separation. This is consistent with the clinical effects of haloperidol (severe EPS) and clozapine (mild EPS). The ratios of MEDs in catalysis and activity for the remaining novel drugs fell between haloperidol and clozapine; this is consistent with the preliminary clinical findings indicating some EPS with each of these compounds. Thus, catalysis remains a suitable rodent model for detecting compounds with EPS liability in humans.


In order to validate the hypothesis that atypical neuroleptics are far more potent in the olfactory tubercle (OT) than in the nucleus accumbens (ACC; Behav. Pharmacol. 3, s18, 1992), we investigated to what extent risperidone, sertindole, olanzapine, ORG 2522 and promethazine, v. all respond claims as atypical neuroleptics, differentially attenuate locomotor activity elicited from the OT and the ACC respectively. First, we used the so-called paw-test to identify the atypical profile of these compounds. (In all these studies, the dose required for enhancing hindlimb retraction time (HRT) was lower than that required for enhancing forelimb retraction time (FRT), underscoring the atypical profile of the tested compounds. Second, the ability of intracerebral administration of the drug (0.5 μL) to attenuate locomotor activity elicited either by dopamine injections (10 μg/0.5 μL) into the OT or by ergotamine injections (1 μg/0.5 μL) into the ACC was compared. Following normal habituation procedures, dose-effect curves (1 μg - 10 μg) were made, using computerized recordings. Using the ratio of the minimum effective doses (OT : ACC), it was found that all compounds were far more potent in the OT than in the ACC. It is concluded that neuroleptics with an atypical profile are far more potent in the olfactory tubercle than the nucleus accumbents; the reverse holds true for classical neuroleptics.

EPILEPSY: ANTICONVULSANT DRUGS—OTHER NEUROTRANSMITTER RECEPTORS


The recently launched anticonvulsant lamotrigine was investigated on TTX-sensitive Na+ currents (I_h) and on spontaneous activity in in vitro models of epileptogenesis, relevant to post-injury, carbamazepine resistant or pentobarbital. All recordings were performed on hippocampal cell cultures using whole cell patch clamp. Sodium currents were activated by membrane depolarization, with the threshold for a greater than 400 μA peak I_h being reversibly depressed in a voltage dependent manner by bath application of lamotrigine. The block of I_h was voltage dependent, with voltage command pulses to 20 mV being increased from 125% (in 2 μM lamotrigine) to 574% (in 20 μM lamotrigine) by lamotrigine. L-V curves of 100% inhibition in 5-μM lamotrigine and 1 (250 μM) reduced lamotrigine to 92% 2% (n=8) and 18.5% (n=7) of control values, respectively. The EC50 value was 28.1 μM at a stimulus frequency of 0.1 Hz. The depolarization of sodium currents by lamotrigine (50 μM) was use dependent, in that raising the stimulus frequency from 0.1 to 5 Hz reduced the peak amplitude of I_h by additional 12% (n=7). In current clamp experiments, the shift in resting membrane potential, high spontaneous activity of neurons as well as paroxysmal discharge pattern observed in the presence of high potassium (7.5 mM) or zero Mg2+ was significantly reduced by lamotrigine, phenytoin and propantabarbital (all tested at 50 μM). This study demonstrates, that lamotrigine and carbamazepine, lamotrigine acts as a voltage dependent sodium channel blocker at therapeutic concentration, which might contribute to the reported reduction of glutamate release.


BW 1205U90 ((cis)-2-(4R-(4,4-dimethyl-3,3-diphenyl-3-piperazinyl)-isobutyryl)-5, 6, 7, 8-benzenethiophenan-2-one hydrochloride) was synthesized as part of a program to develop antipsychotics with reduced liability for extrapyramidal side effects (EPS). It bound to 5-HT4 receptors (IC50 = 0.9 nM), more readily than to dopamine D2 receptors (IC50 = 1.6 nM) and also bound to 5-HT4 and adrenergic α1 receptors potently. In mice, BW 1205U90 antagonized climbing (efficacy) and stereotyped behavior (potency) with apomorphine with pEC50s = 674 and 74 mg/kg p.o., and 0.04 and 0.6 mg/kg s.c., respectively. The ED50 for induction of catalepsy (which predicts EPS) was 58 mg/kg p.o. Head switches in mice induced by the serotonin 5-methoxytryptamine-aminergic were antagonized with ED50 = 2.0 mg/kg p.o. and 0.03 mg/kg s.c. In rats, BW 1205U90 blocked apomorphine-induced circling in subjects with unilateral 6-hydroxydopamine lesions of the striatum (ED50 = 1.1 mg/kg p.o.) and antagonized apomorphine-induced stereotyped (ED50 = 3.3 mg/kg p.o.). Apomorphine-induced disruption of prepulse inhibition of acoustic startle reflex was antagonized at 0.2 mg/kg p.o. and conditioned avoidance response was inhibited with an ED50 = 0.05 mg/kg s.c. Shaking behavior induced by the serotonin precursor 5-hydroxytryptophan, was antagonized (ED50 = 0.8 mg/kg p.o.). BW 1205U90 antagonized 5-HT2-mediated effects more potently than D2-mediated effects. It was potent to tests predictive of antipsychotic efficacy, but much less potent in tests for EPS.
Further evidence that serotonin plays an anticonvulsant role in genetically epilepsy-prone rats (GEPRs). Q.S. Yan, P. C. Jibe, and J. W. Dailey, Department of Basic Sciences, University of Illinois College of Medicine at Peoria, Peoria, Illinois 61656.

Preceding reports in this laboratory indicate that fluoxetine, a selective serotonin uptake inhibitor, has anticonvulsant effects in genetically epilepsy-prone rats (GEPRs). Also, the protection against audiogenic seizure following the administration of fluoxetine appears to be selectively correlated with enhanced serotonergic transmission (Dailey et al., 1992; Yan et al., 1994). An important question raised by these findings is whether the anticonvulsant action following the administration of the serotonin uptake inhibitor in GEPRs is peculiar to fluoxetine. This study was designed to evaluate further the role of serotonin in regulating susceptibility and/or severity of audiogenic seizures in GEPRs. For this purpose, sertraline, another highly selective and potent inhibitor of serotonin uptake, was studied. Sertraline was found to be comparable to fluoxetine in preventing audiogenic seizure in GEPRs. These findings provide further support for the concept that serotonin plays an anticonvulsant role in GEPRs.


Gabapentin (GBP), 1-(aminomethyl) cyclohexane-acetic acid, is a new anticonvulsant drug which is used clinically in the United Kingdom and the United States, but its mode of action is unknown. The antiepileptic properties of GBP were initially predicted because it is a structural analogue of the inhibitory amino acid neurotransmitter γ-aminobutyric acid (GABA). However, it lacks agonist and antagonist properties at both GABA_A and GABA_B receptor sites, and does not appear to block GABA uptake. GBP has been shown to increase the apparent rate of synthesis of GABA in several brain regions, and enhanced promoted release of GABA following GBP treatment has been observed in neonatal rat hippocampus. Here we present electrophysiological experiments in the rat hippocampal slice preparation that GBP treatment enhances the release in excitatory postsynaptic potentials (EPSPs) elicited by promoted GABA release with nipeptic acid (NPA). NPA blocks both uptake of GABA and promotes release by heteroexchange through the GABA transporter. GBP had no effect on the normal excitability properties of the hippocampus. We suggest a novel mechanism for the antiepileptic properties of GBP whereby there is enhanced nonvesicular release of GABA possibly through reverse operation of the GABA transporter at times of intense electrical activity.


D-23129 (N-2-amino-4-4(4-fluorobenzylamino)-phenyl)proionic acid ethyl ester, Asta Medica AG, Germany, ADD230001) is a promising new anticonvulssant compound under investigation in the Antiepileptic Drug Development (ADD) Program. Recently, this compound was also shown to have some unique properties in suppressing in vivo epileptiform activity in rat hippocampal slices (W.D. Yonekawa et al., Soc. Neuropsy. Abstr. 17:117). Here we present electrophysiological experiments in the rat hippocampal slice preparation that GBP treatment enhances the reduction in excitatory postsynaptic potentials (EPSPs) elicited by promoted GABA release with nipeptic acid (NPA). NPA blocks both uptake of GABA and promotes release by heteroexchange through the GABA transporter. GBP had no effect on the normal excitability properties of the hippocampus. We suggest a novel mechanism for the antiepileptic properties of GBP whereby there is enhanced nonvesicular release of GABA possibly through reverse operation of the GABA transporter at times of intense electrical activity.

Way10135 antagonizes the serotonin-mediated inhibition of epileptiform activity in hippocampal CA1 neurones. Delanty Salgado*, and Karim A. Alkudhi, Department of Pharmacology and Neuroscience, University of Houston, Houston, TX 77204-5515 USA.

Previous studies conducted in our laboratory have shown that serotonin inhibits epileptiform activity (Salgado and Alkudhi, Soc. Neurosci. Abstr., 764.4, 1993). In addition, experiments done thus far suggest that the serotonin-mediated inhibition of epileptiform discharge is due to activation of the 5-HT1A receptor subtype. The purpose of this study was to provide more conclusive evidence regarding the involvement of the 5-HT1A receptor in the inhibition of epileptiform activity. Thus we used the new, selective 5-HT1A receptor antagonist, WAY10135. We employed conventional electrophysiological techniques for intracellular recording from CA1 neurones in the rat brain slice preparation. Initial experiments demonstrated that WAY10135 (10 pM) prevents the serotonin-mediated hyperpolarization, decrease in membrane resistance and current-induced action potential bursts. Furthermore, the serotonin-mediated suppression of bicuculline-induced epileptiform bursts was also antagonized in the presence of WAY10135. The results obtained thus far strongly suggest that activation of 5-HT1A receptors in hippocampal CA1 neurones can suppress epileptiform activity. This study suggests that treatment of epilepsy may be possible with agents selective serotonin 5-HT1A receptor sites on hippocampal CA1 neurones.


The effect of elevated GABA levels on the gial enzyme glutamine synthetase (GS) was examined in rat brain. Repeated daily s.c. injections of the GABA-transaminase inhibitor, gamma-vinylGABA (GVG), an anti-epileptic medication, produced a gradual decline in brain GS activity. After 21 days, cortical GS activity was reduced 36% with 150 mg/kg per day and 9.5% with 30 mg/kg per day compared to saline-injected rats. Cerebellar GS activity was reduced by 22% with 150 mg/kg and unchanged with 30 mg/kg. Skeletal muscle and liver GS activities were unaffected. Cortical GABA levels were increased by 170% after 150 mg/kg for 21 days but were unaffected at 30 mg/kg. Glutamine and glutamate levels were significantly reduced only at the 150 mg/kg dose. GS mRNA levels were unaffected, as shown by Northern analysis. Levels of GABA in liver were maximally elevated even after this short dosing interval. The delay in the effect of GVG on GS may be related to the slow turnover of this enzyme in vivo. The glutamine cycle, which provides substrate for neuronal GABA and glutamate synthesis, may be down-regulated by chronically elevated GABA. The reduction in GS activity appears to decrease brain glutamine production and thereby may limit transmitter glutamate synthesis and contribute to the anti-epileptic effect of GVG.

Novel mechanism of action of anticonvulsant alkyl-substituted thiobutyrolactones: reduction of neuronal Ca current. J.A. Fernandelli*, D.A. Gross*, D.C. Cowey*, and R.W. Subpals*, Jr. Dept. of Neurology 2 and Pharmacology 1, The University of Texas Health Science Center, St. Louis, MO, University of Minnesota, Minneapolis, MN.

Alkyl-substituted thiobutyrolactones (TBLS) produce either convulsant or anticonvulsive effects by modulation of the picrotoxin (PTX) GABA_A receptor. Because of structural similarity to ethosuximide, an antiepileptic drug that blocks T-type Ca currents, we tested the effects of the anticonvulsant a-ethyloctanal-thiobutyrolactone (a-EMBL) on Ca currents of cultured DRG and nociceptive neurones.

Whole cell Ca currents were evoked at +10-20 mV in media which blocked Na and K currents, with 5 mM Ca as the charge carrier. Effects on Ca channel subtypes were assessed (a-EMBL) and a-conotoxin GIVIA (N-type) and a-conotoxin IVIA (P-type). a-EMBL (500 μM) had a voltage-dependent effect: high-threshold DRG currents were reduced ≤ 10% when evoked from -40 mV, but by 30-50% when evoked from -40 mV. Current inactivation was increased, with a greater effect on currents evoked from more polarized potentials. Nociceptor currents were similarly affected. In DRG neurones, 10 μM a-conotoxin GIAB reduced Ca currents ~50% and 500 nM a-conotoxin IVIA reduced currents ~15%; 10 μM nifedipine reduced currents 10-15% at -40 mV and 40% at 40 mV. None of these, used alone, significantly affected the action potentials evoked by a-EMBL. These data suggest that a-EMBL may have a second mechanism of action, reduction of neuronal Ca currents. This is relatively non-specific for channel subtypes and may mediate neuronal excitation.

Supported in part by NS19613 (RAG) and NS14858 (DFC, JAP).
EPILEPSY: ANTICONVULSANT DRUGS—OTHER NEUROTRANSMITTER RECEPTORS

**669.9**

**RHOBITION OF MAXIMAL ELECTROSHOCK (MES) SEIZURES BY A1 ADENOSINE RECEPTOR AGONISTS.** J.B. Weizer and S. Zimling, Gernisa, etc., San Diego, CA 92121.

Adenosine receptor agonists exert anticonvulsant activity in a variety of animal models, including those involving chemically-induced seizures, audiogenic seizures, and kindled seizures. However, Little information exists concerning their activity on the maximal electroshock seizure. Since the efficacy profile in such seizure models are often believed to predict efficacy in clinical seizure types, we investigated the effect of adenosine agonists in MES seizures, as a way to model seizure induced by s.c. pentyleneetrazole (PTZ). We also investigated effects on blood pressure, body temperature, and motor performance. Extensive criteria (80 Hz, 0.2 seconds) was delivered via coronal electrodes at 150 V in rats and 50 mA in mice at 20 min after i.p. administration of each agonist. The A1 receptor agonists cyclopentyladenosine, R-phenylisopropyladenosine, 2-chloroadenosine, and N6-cyclohexyladenosine, dose-dependently inhibited MES seizures in both species. Respective ED50 values (mg/kg) for MES in rats were 2.3, 3.7, 3.6, 5.7 and 10.1. Respective ED50 values for MES in mice were 1.7, 3.6, 13.4, 8.2, and 73.8. The A2 selective agonist CV18008 was ineffective at doses of up to 10-50 mg/kg. The A1 agonists also inhibited PTZ seizures with respective ED50 values in brain, 4.5, 11.0, and 9.1 (mg/kg). The A1, 1.7, 0.6, 5.7, 3.2, and 0.1 (mg/kg). All of the A1 agonists induced substantial kinetic or static effects at anticonvulsant doses. In addition, the ED50 dose (2.3 mg/kg) of cyclopentyladenosine in conscious rats decreased blood pressure and heat rate by approximately 60% and decreased body temperature by 4-6°C.

These results show adenosine receptor agonists possess anticonvulsant activity in the maximal electroshock model in rats and mice, indicating potential utility of an adenosine agonist approach in generalized tonic-clonic seizures in man. However, the clinical efficacy of direct A1 agonists will be limited if similar side effects are found in man.

**669.10**

**THE PHENYLCARBAMIC ACID ESTER D-23129 IS HIGHLY EFFECTIVE IN EPILEPSY MODELS FOR GENERALISED AND FOCAL SEIZURES AT NONTOXIC DOSES.** C. Töber, C. Rundfeldt*, A. Rostock and R. Barths, Arzneimittelwerk Dresdon GmbH, Department of Pharmacology, Meilner Str. 191, 01445 Kadele, FRG.

Complex partial seizures comprise the major uncontrolled seizure type in adult patients with epilepsy. New anticonvulsants should not only reduce seizure severity but also suppress focal seizure activity for full seizure protection. The goal of this study was to demonstrate that D-23129 is capable to suppress focal seizure activity at nontoxic doses. The broad efficacy of D-32441, the hydrazide derivative of D-23129 (N-[2-Aminomethyl-4-(4-fluorobenzyl) phenyl]carbamic acid ester), was shown previously (Nickel et al, Epilepsia 34, Suppl. 2, 95, 1993). We could now demonstrate that D-23129 and D-23128 are equipotent to the toxic extension of hind limb in the maximal electroshock test in mice (ED50 18.2 and 17.4 mg/kg i.p., respectively); both compounds also nearly doubled the threshold for clonic seizures in the i.v.-model after administration of 15 mg/kg i.p. and suppressed audiogenic seizures in DBA/2 mice at 3 mg/kg. D-23129 reduced not only kindled seizure parameters after supramaximal stimulation but also increased the threshold for induction of afterdischarges indicating a suppression of focal seizure activity in a dose dependent manner. After oral administration of 10 mg/kg 60 min prior to testing all animals were seizure protected if stimulated with double the individual seizure threshold. The afterdischarge threshold was increased to 408 and 597% of control threshold after 10 and 15 mg/kg respectively. After 15 mg/kg all seizure parameters were significantly reduced after suprathreshold stimulation and no motor impairment was present. First electrographical data indicate that part of the effects of D-23129 is mediated through effects on voltage gated Na+ and Ca2+ channels. The results make D-23121 a promising candidate for treatment of generalized and complex partial seizures.

**669.2**

**DEGENERATIVE DISEASE: ALZHEIMER’S—BETA AMYLIOD XI**

**669.3**


Deposition of the 4 KD Aβ peptide, by proteinolytic cleavage from the amyloid precursor protein (APP), in brain is a neuropathological hallmark of Alzheimer’s disease (AD). Expression of full length human APP in brains of animals should provide a model system for studying proteinolytic processing of APP in vivo. These animals also serve as a basis to address parameters required for the development of B-amyloid related neuropathologies in man. We have generated transgenic mouse and rat lines harboring full length human APP transgenes containing native splice site and genetic mutations linked to familial AD (Aβ1-42, ΔNle233/234) and hereditary cerebral hemorrhage with amyloidosis-Dutch type (Aβ693). Human APP transgene RNA and protein occurs predominantly in brain. In situ hybridization studies show human APP enriched in neurons. Evidence for proteolytic cleavage of human APP in mouse brain which is required for Aβ production has been obtained using antibodies specific for human APP fragments. Recent evidence has implicated apolipoprotein E in the pathogenesis of AD. Apolipoprotein allele E4 cosegregates at high frequency with late-onset and sporadic AD. To study the role of apoE in amyloid deposition we have generated transgenic lines expressing both human apoE and Aβ. Characterization of these lines and results from our ongoing search for amyloid-related neuropathologies in a transgenic mouse and rats will be presented.

**669.4**

**APOLIPOPROTEIN E REGULATES APP LEVELS.** Benjamin Wolozin*, Robert Carbon, Yongqiang Chen, Robert Culver, Todd Futterer, Tony Sunderland, and Robert Greenberg. Lab. of Clinical Science and Lab. of Biochemical Genetics, National Institute of Mental Health, Bethesda, MD 20892.

Recent studies have shown that the apolipoprotein E (apo E) allele, e4, is an important risk factor for Alzheimer’s disease. Previous studies showing that Apo E binds to B-amyloid, in vitro, and associates with neuritic plaques, in vivo, also the risk may be due to a genetic association between B-amyloid and apo E (W. J. Strittmatter, et al, 1993 PNAS 90:8086). However, the mechanism for this association is unclear because the affinity of Apo E for B-amyloid is low. We have shown that apolipoprotein E is a potent regulator of APP levels in cell culture, being active at levels as low as 30 nM. Addition of apolipoprotein E or HDL to PC12 cells grown in serum-free medium increased the expression of both secreted and cellular amyloid precursor protein (APP) in a dose-dependent manner by up to 65%, with an IC50 of 30 nM. Blockade of protein processing with 1 mM brefeldin A, 60 mM chloroquine or 10 μg/ml leupeptin prevents this, which suggests that internalization and catabolism of APP or Apo E is necessary for this process. These data point to a physiologic role for apo E in the regulation of APP and suggest that binding of apo E to APP may be the first step in the association between Apo E and B-amyloid.

Two apolipoproteins, apoE and apoJ, have been recently shown to be associated with Alzheimer's disease (AD). It has been proposed that apoE is an AD pathophysiological chaperone protein, acting to modulate and/or stabilize a β-plaleted sheet structure in AD. Consistent with this hypothesis, there is a linkage between apoE4 isotype and late onset Alzheimer's disease. On the other hand, apoJ has been shown to be complexed to Aβ in cerebrospinal fluid. Aβ, apoE and apoJ are present in senile plaques; however, their origin is not known. We have studied the behavior of these three components at the BBB level. An "in vivo" brain perfusion technique and capillary depletion method were used to determine cerebrospinal fluid and BBB transport of [125I] labeled apoJ, apoE and synthetic Aβ1-40 in guinea pigs. A saturable specific binding and transcellular BBB transport were demonstrated for all components although both apolipoproteins exhibit different BBB transport behaviors. After 10 min perfusion, the volume of distribution for apoJ was 4.5 times higher than for apoE. Pre-perfusion with a very low dose of cold apoJ completely blocks the transport, indicating a high affinity interaction.

669.7 BRAIN CLUSTERIN (AP0-J) AND ITS INTERACTION WITH β-AMYLOID (AB) (A) G.M. Pavlides*, T. Oda, H.A. Johnson, C.E. Finch. Andrus Gerontology Center Neurogerontology Division and Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.

The senile plaque contains aggregations of β-amyloid (AB). In addition to aggregated AB, senile plaque contains other proteins that may be pertinent to neurodegeneration, particularly complement proteins and the putative complement inhibitor, clusterin. We purified clusterin from Alzheimer's disease (AB) cerebral cortex by column chromatography (DEAE, antibody affinity and HPLC size-exclusion). Brain clusterin presented slightly smaller species about 35-40 kDa vs. purified serum clusterin (39-42 kDa), under reduced conditions. In the inhibition of complement-mediated hemolysis, brain and serum clusterin were indistinguishable. Aggregation of AB1-42 was assayed by centrifugation with [125I]-AB1-42 and gave 30% sedimentable Aβ (48 hrs, 29°C, pH 7.0). Clusterin showed a concentration-dependent inhibition of AB sedimentation. Supported by Alzheimer's Association grant to GNP and by grants to OEF from NIA AG-7909 and Sankyo Co.

669.8 ALZHEIMER'S Aβ AND APOLIPOPROTEIN J INTERACTION. E. Matsubara, B. Frangione, J. Ghiso*. Dept of Pathology, NYU Medical Center, New York, NY, 10016.

Peptides with the same amino acid sequence as Alzheimer's amyloid B (AB) exist as a normal soluble protein (sAB) in biological fluids. Hence, a key question is what factors alter sAB in the disease state, promoting conformational changes, amyloid fibril formation and cell death. Biochemical and immunohistochemical studies have shown 1) sAB and ApJ bind sAB under different conditions in vitro, and 2) sAB is complexed to ApJ in vivo. We have further characterized the interaction ApJ-sAB with purified ApJ and immobilized Aβ1-40 on a solid phase on capture ELISA experiments. The binding ApJ-Aβ1-40 was saturable and specific. When the preformed complex was offered to Aβ1-40 complete binding inhibition was achieved, indicating complex stability in physiologic solutions. Solid phase competitive inhibition assay using other plasma/cerebrospinal fluid proteins with demonstrated avidity for ApJ (ApoE3, ApoE4, α1-antichymo-trypsin, Transthyrein, Vitronectin) showed that none of these completely displaced apoJ from the complex up to 100 molar excess. Only ApoJ at high molarity (approximately 60 molar excess over apoJ) partially competes with ApJ for Aβ. The data support the notion that the association ApJ-Aβ may play a role in the transport and delivery of sAB.
660.11

BIOCHEMICAL AND IMMUNOBIOCHEMICAL STUDIES OF THE ROLE OF ALZHEIMER'S DISEASE-ASSOCIATED PROTEINS IN THE PATHOGENESIS OF NEURODEGENERATION. J. D. Selkoe and J. H. Kim. Department of Neurosciences, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

660.12

BINDING OF SOLUBLE β-AMYLOID PEPTIDE IN VITRO AND IN VIVO. A. Golobek, M. Marques, L. Koji, J. Ghio, J. Herbert*, B. Frangione, T. Wisniewski. Dept of Pathology and Neurology, New York Medical Center University, N.Y., 10016.

660.13


660.14


660.15

AN ELISA ASSAY TO DETECT A DOPAMINE RELEASING PROTEIN (DARP) AND POTENTIAL CLINICAL APPLICATIONS. S. Kohneman and V.D. Ramig. Dept of Molecular and Integrative Physiology, Univ of Illinois Urbana, IL 61801.

660.16

TREATMENT WITH A SYNTHETIC 35aa PEPTIDE FROM THE N-TERMINAL SEQUENCE OF DARP PARTIALLY RESTORED DA LEVELS IN MPP+ LESIONED RATS AND DRastically REDUCED AMPHETAMINE-INDUCED ROTATION. Ramirez VP*, Phillips S. Kim S., Department of Molecular and Integrative Physiology, Univ. Illinois, Urbana, IL 61801, USA.

670.1

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670.2

TREATMENT WITH A SYNTHETIC 35aa PEPTIDE FROM THE N-TERMINAL SEQUENCE OF DARP RESTORED DA LEVELS IN MPP+ LESIONED RATS AND DRastically REDUCED AMPHETAMINE-INDUCED ROTATION. Ramirez VP*, Phillips S., Kim S., Department of Molecular and Integrative Physiology, Univ. Illinois, Urbana, IL 61801, USA.
**670.5** UNILATERAL INTRACAROTID ADMINISTRATION OF MPTP IN NONHUMAN PRIMATES: EFFECT ON DIFFERENTIAL SUBSTANTIA NIGRA CELL LOSS, R. Assenti, K.D. Terry, N. Oreski*, RAE Bakay, Department of Neurosurgery, Emory University, Atlanta, GA 30322.

Behaviorally, the similarities between the MPTP non human primates model and Parkinson's disease (PD) are quite striking. MPTP's neurotoxicological effects (specifically loss of dopaminergic neurons in the substantia nigra have been studied in a manner similar to that used to study human PD, and meaningful comparisons have been difficult to obtain. In this study, rhesus (Macaca mulatta) monkeys (N=12) with MPTP administration (1 mg/kg by IP injection on 1-20 days) and unilateral intracarotid (left side) injections of MPTP (0.5mg/kg over a 20 minute period). The monkeys were observed for at least 6 months with saline on TBI (10 months) and then sacrificed. The monkeys were tested for various dopamine related parameters (range: 1-3 years old). Normal monkeys (N=9) with ages ranging 1 to 24 years of age were examined in a similar fashion. Serial sections of the midbrain were obtained and immunocytochemically processed for tyrosine hydroxylase (TH). From these sections, the number, size, and nuclear diameters and cell volume were measured in A8, A9 (dorsolateral, dorsomedial, ventralateral, ventromedial), A10 parasympathetic nucleus and A10 total areas using a computerized image analyzer and corrected according to the Abercrombie formula. No difference in cell counts was observed in comparing the left and right sides of the normal monkeys. A trend in cell loss according to age was observed in both normal (dorsolateral A9) and MPTP treated (ventralateral A9) animals. The most consistent results were obtained using the A9 (ventralateral) area. The relative mean TH cell loss in the A9 area (pars compacta) compared to the mean values observed in the normal monkeys was: dorsolateral A9: -82.3%; dorsomedial A9: -73%; ventralateral A9: -90.1%; ventromedial A9: -96.6%. Age: total A9: -42.2% (p<0.000). Relative cell loss in A8 was 68% and in A10 (ventral medial area) total: 74% with a p<0.05. There was no significant cell loss observed in A10 parasympathetic nucleus. The analysis of our data demonstrates that the greatest amount of TH cell loss occurred in the raphe nucleus, MPTP, occurs in A9, and most severely in the ventralateral area of A9. Importantly, this is an area that has been shown to be most affected in human Parkinson's disease. Supported by USPHS Grant RR00165 and NINDS NS253430.


We hypothesized that MPP+, accumulated in dopaminergic synaptic vesicles during repeated treatment with subtoxic doses of MPTP, could be released by high subtoxic nigral toxin, yielding a toxic concentration of MPP+. Animals were treated with MPTP iv. 3 times/wk at 0.1 mg/kg for 8 weeks (low dose; total dose 1.24 mg/kg) or at 0.1 mg/kg for 4 wks (high dose; total dose 3.6 mg/kg). At the end of dosing, one of the high dose group was sacrificed. Remaining high and low dose animals were challenged with MPTP (25 mg/kg sc.) 3 times per day for 4 days. Neuropeptide markers were assessed. Controls got 8 saline, TBZ, then 4 wks recovery. The repeated treatment pattern caused a dose-related decrease in striatal dopamine (DA) 4 wk after treatment and a loss of striatal DA. The substantia nigra pars compacta (SNc) markers were largely intact, but swollen, degenerating cells were evident. The high dose animals showed >90% reduction in striatal DA and marked SNc cellular pathology (e.g. swollen, distorted or pyknotic neurons with disintegrating processes). Animals in the high dose group sacrificed immediately after the 2 month dosing period showed a modest reduction (38%) in striatal DA and minimal damage to the SNc. Challenge with TBZ following subchronic low dose MPTP caused ptox, but did not enhance the toxicity of MPTP. These observations suggest that degeneration progressed following the end of MPTP dosing, but was not enhanced by TBZ treatment. Adrenal MPP+ levels were not related to cumulative MPTP dose or survival time. While all treatment groups receiving MPTP had high levels of adrenal MPP+, there were no differences between groups, suggesting that 1) accumulation was limited, 2) clearance of accumulated MPP+ from adrenal was very slow and 3) treatment did not cause release of MPP+ from chromaffin granules, its presumptive storage site.

**670.7** 3HFLUORITRAZINE BINDING TO GABA RECEPTORS IN THE EFFERENT PATHWAYS OF THE STRIATUM OF MPTP MONKEYS TREATED CHRONICALLY WITH A D2 AGONIST (U-91356A) OR L-DOPA: RELATIONSHIP WITH DEVELOPMENT OF OPUترتيبUSE, D. Chavrol, P. Majolet, E. Declerck, J. Gegard, J. Delbeuck, J. Hirsch, and T. Di Paolo, 670.7. School of Pharmacy, 2Department of Pharmacology, Laval University, Quebec, CANADA, G1K 7P4.

Dyskinetic monkeys have the same side effects (frequent or impulsive dopaminergic treatment) as Parkinsonians and in MPTP monkeys. Most of the basal ganglia neurons are gabaergic and are probably implicated in the pathogenesis of the dystonia. This is, the gaba/benzodiazepine receptor complex was quantified by autoradiographic procedures using 3Hfluoritrazine (3HFTZ) binding. Following continuous or intermittent D2 agonist treatment with U-91356A (Uj) or intermittent L-DOPA treatment. Animals treated intermitently were sacrificed after three days, a time when no dyskinesia was induced while animals treated continuously with U-91356A did not. 3HFTZ binding in the striatum, internal (GIp) and external segments of globus pallidus, subthalamic nucleus and substantia nigra were investigated. 3HFTZ binding in nigral dyskinesia was induced by continuous treatment with U-91356A without L-DOPA treatment. The most interesting change was an elevation of 3HFTZ binding in the GIp of animals treated with U-91356A (Gi) or levodopa continuously developing dyskinesia (+38% and +36% vs MPTP untreated, respectively, p<0.05). These results suggest supersensitivity of the GIp to gaba/benzodiazepine receptor stimulation. Supported by the MRC of Canada and the Parkinson Foundation of Canada.


Loss of midbrain dopaminergic neurons is the pathological characteristic of Parkinson's disease (PD) in human and neurototoxicity in monkeys. A decrease in tyrosine hydroxylase (TH) mRNA content in the striatum of the substantia nigra in the surviving neurons in the midbrain from PD patients. Yet, because all patients are treated by levodopa, it is difficult to determine if this decrease is due to dopaminergic denervation or to levodopa therapy. The present study was aimed to analyze by quantitative in situ hybridization the effect of MPTP intoxication and levodopa therapy on the mRNA encoding for TH in four groups of Macaca fascicularis monkeys: normal control animals, MPTP-treated animals with moderate or severe parkinsonian symptoms, and levodopa-treated parkinsonian monkeys. Midbrain sections were hybridized with a 32P-labeled oligonucleotide riboprobe for TH mRNA. Quantification of mRNA expression at the cellular level using computerized image analysis revealed a decrease in TH mRNA expression in dopaminergic neurons from normal, MPTP-intoxicated and levodopa-treated monkeys as compared to control monkeys without statistically significant differences between the three groups. Similarly, TH protein content measured in the same animals also decreased. These results suggest that dopaminergic denervation induces a decrease in TH mRNA and protein expression which is not reversed by levodopa therapy.
DEGENERATIVE

with Cholinergic... 

leptorhine monkeys, the ability of CPP, a competitive NMDA antagonist, and NBQX, a competitive NMDA antagonist, when administered alone or in combination with a sub-threshold dose of a-pompholine (APO), to reverse HAL-induced EPS. Subjects were opossum monkeys that were sensitized to acute EPS by once weekly administration of haloperidol (HAL). Individual EPS signs were rated by non-blinded observers on a three point scale, totaled at each time point for each monkey, and group means derived. Test agents were administered 3 hr after administration of HAL, a time at which EPS signs were well established and stable. Ratings were made every 15 min for 1 hr after drug challenge and then at 30-45 min intervals for the next 2 hr. APO, given i.m., produced a dose-related reversal of all EPS signs. No reversal of the HAL-induced EPS was produced by NBQX, CPP or the combination of NBQX and CPP, given s.c. Neither NBQX nor CPP potentiated the ability of a sub-threshold dose of APO to reverse the HAL-induced EPS. These data suggest that glutamate antagonists would not be effective in ameliorating AP-induced EPS and question their role in the treatment of PD. Further experiments are evaluating the effects of treatments designed to increase the brain levels of NBQX on these interactions.

ALTERATIONS OF PALLIDAL CHOLINERGIC ACTIVITY IN MPTP TREATED MONKEYS. EFFECT OF DIHYDRO-alpha-ERGOCRYPTINE (DEK) 

The neurotoxic MPTP induces Parkinson(PD)-like symptoms in human and non-human primates. Recent studies have shown that dopamine loss in PD patients is not well understood and it is likely to be related to PD-associated motor disturbances (Campbell and Dill, Exp.Neurol., 42, 1974). The aim of this investigation was to evaluate in a small number of extrapyramidal brain regions, the short term consequences of MPTP administration on the cholinergic enzymes (choline acetyltransferase, CACh, and acetylcholinesterase, ACEh) and on pyruvate dehydrogenase complex (PDHC) which catalyzes the oxidative decarboxylation of pyruvate to acetylcoenzyme A and links cholinergic and energy metabolism. A treatment with dihydro-alpha-ergocryptine (DEK), a D2 receptor agonist was also performed. This ergocryptine improves motor performances PD patients (Martignoni et al., Clin.Neuropharmac, 14, 1991). Monkeys, intravenously administered with MPTP at the dose of 0.3 mg/kg for 5 consecutive days, develop a severe PD-like syndrome. Cholinergic enzyme activities are increased in the internal segment of the globus pallidus (GPH) and into a lesser extent in the external globus pallidus (GPs). Cholinergic activities are not significantly affected in the caudate and putamen nor in the frontal, parietotemporal, occipital cortices and cerebellum. The treatment of the animals twice daily for two weeks with DEK starting 5 days before the first MPTP administration counteracts the neurotoxin-induced alteration in the internal pallidum and ameliorates some motor related parkinsonian symptoms.


MPTP provides selective destruction of dopaminergic (DA) neurons in the substantia nigra (SN). Previous studies have shown that riluzole (2-amino-6-

1-1,3-thiazol-2-yl)-5-phenyl-3,4-dihydropyridin-2(1H)-one, a drug which interferes with glutamatergic neurotransmission, has a neuroprotective action in rodent models of cerebral ischemia. Effects were performed on two neurotransmitter systems (RI and RII). Both monkeys received a single injection of MPTP (0.6 mg/kg) in the right internal caudate. RI was injected with saline one hour before and six hours after the injection of MPTP. From days 2 to 30, it received further administration of saline. IRI injected saline one hour before and six hours after the injection of MPTP. From day 2 to 30, RI received a single daily injection of riluzole (0.4 mg/kg) in the right internal caudate. RI was injected with saline one hour before and six hours after the injection of MPTP. From days 2 to 30, it received further administration of saline. IRI received saline one hour before and six hours after the injection of MPTP. From day 2 to 30, RI received a single daily injection of riluzole. Both monkeys were examined clinically and muscular rigidity was quantified by electromyography. Then, they were administered with MPTP, brainstem and rigidity were absent. When injected daily in RI, riluzole significantly reduced bradykinesia and rigidity. RIVADA rum, clinically assessed, were indexed to an index of DA use, increase of 8% after MPTP treatment in RI. Interestingly, the neuroprotective effect of riluzole with riluzole in RI increased more markedly this ratio (92%). That, a neuroprotection coupled to a facilitation of DA release may explain the behavioral effects reported with riluzole treatment. These results show that riluzole would potentiate both neuroprotective and palliative effects in a primate model of Parkinson's disease.


Magnetic resonance imaging (MRI) has recently been used to map the scalar diffusivity (D) of water in tissues. In brain ischemia, D is decreased when ventilatory MRI is still uninformative. It has been recently proposed that the transmission of effective diffusion (Tr(Deff)) measured by diffusion tensor imaging, more accurately reflects the mobility of water in tissue (1). We evaluated the utility of Tr(Deff) to detect selective neuronal lesions such as the degeneration of the substantia nigra pars compacta (SNc). SNc neurons are affected in the neurotransmission following MPTP systemic administration. Cynomolgus monkeys were administered with MPTP (0.5 to 1.5 mg/kg i.v. weekly) and scanned with MRI before, immediately after their first MPTP administration and then at 1 week after each MPTP injection. Each scan consisted of the acquisition of 14 diffusion weighted coronal images from which both Tr(Deff) and T2 weighted signal intensity maps were calculated. In the 2 stable parkinsonian animals (cumulative dose of 3-3.5 mg/kg of MPTP) studied so far, Tr(Deff) was decreased by 22% in the doro-lateral putamen and by 6% in the caudate nucleus. No changes were observed in other brain structures. Tr(Deff) alteration persisted in one animal scanned 44 days after the last MPTP injection. These findings are consistent with previous studies. These data of this ongoing study suggest that Tr(Deff) could provide “in vivo” information on selective brain damage.

EFFECTS OF THE FULL Dopamine D-1 Receptor Agonist Dihydrexidine on Cognitive Function and Motor Activity in MPTP-Treated Monkeys. J. W. Johnson, J.S. Schneider, Dept. of Neurology, Hahneham University, Philadelphia, PA 19102.

Dopamine D2 receptors have been assigned a major role in regulating motor function, based primarily on rodent and human studies with D-2 agonists and antagonists. Dihydrexidine (DIH) is a potent and selective D1 agonist SKF 38393 on parkinsonian monkeys and humans. However, there are still questions concerning the potential role of D-1 receptors in treating parkinsonism. The discovery of full efficacy D-1 agonists should help to answer questions concerning the therapeutic potential of D-1 receptor stimulation. In the present study, we used the D-1 agonist dihydrexidine and the potential of this agent to induce rotational asymmetry in hemiparkinsonian monkeys and to correct cognitive deficits resulting from chronic low dose MPTP exposure. Dihydrexidine (0.3 to 0.9 mg/kg), administered to 3 hemiparkinsonian monkeys, caused a dose-dependent increase in contralateral rotations that could be blocked by the D-1 antagonist SCH 23390 but not by the D-2 antagonist raclopride. Dihydrexidine also caused a dose-dependent reversal of the number of cognitive errors made by 3 chronic low dose MPTP-treated monkeys during delayed response performance. This effect could also be blocked by the D-1 antagonist SCH 23390. These results suggest that D-1 receptor stimulation may be therapeutically effective in treating parkinsonism. Additional studies are needed to further compare the therapeutic efficacy of selective D-1 and D-2 receptor agonists in parkinsonian primate models. Supported by NIH grant MH-46531.
**670.15**
CHRONIC GM1 GANGLIOSIDE ADMINISTRATION REVERSES COGNITIVE DEFICITS AND DELAYS ONSET OF MOTOR SYMPTOMS IN A SLOWLY PROGRESSING PARKINSON MODEL IN MONKEYS. L. G. Colemant, A. D. Poeggel and J. S. Schneider. Dept. of Neurology, Hahnemann University, Philadelphia, PA 19102.

We have previously shown that monkeys administered for 6 to 8 weeks after the last of several MPTP injections, can reverse motor deficits and partially restore striatal dopamine levels in monkeys with severe Parkinson's disease (1989, 1992). We have now demonstrated that GM1 might be most effective as an anti-Parkinson therapy if administered early after diagnosis, when there is still considerable loss dopaminergic function. This study was conducted to assess the potential of long-term GM1 treatment as a means of slowing the progression of parkinsonism. Five monkeys, previously receiving low doses of MPTP for 24 weeks. These monkeys developed cognitive deficits, followed by mild motor deficits. At this stage, judged to be analogous to early parkinisonism, monkeys were randomly assigned to receive either saline or GM1 injections (30 mg/kg) while all monkeys continued to receive the same doses of MPTP. The monkeys receiving GM1 showed significant improvement in cognitive task performance by the end of the first 8 weeks of treatment that has been maintained over time while the saline-treated animals have continued to show increasing task performance deficits. Saline-treated animals are also showing a trend toward a progressive decrease in motor ability not apparent in GM1-treated animals. These results suggest that early intervention with GM1 ganglioside treatment may exert both symptomatic and preventive effects in Parkinson's disease patients. Supported by the F.M. Kirby Fund and Fida Pharmaceuticals.

**670.16**
EFFECTS OF GM1 GANGLIOSIDE TREATMENT ON DOPAMINE RELEASE AND REUPTAKE IN MPTP-TREATED MICE. D.S. Rothblat* and J.S. Schneider. Dept. of Neurology, Hahnemann University School of Medicine, Philadelphia, PA 19102.

Both in vivo and in vitro studies have suggested that GM1 ganglioside treatment may reduce the severity of dopaminergic pathology in MPTP/MPP+-damaged dopamine (DA) neurons. Recent data also suggest that GM1 may enhance DA synthesis in residual neurons. This study measured striatal dopamine levels in mice receiving GM1 and saline during MPTP administration and then saline or GM1 for another 3 weeks. MPTP administration resulted in a decrease of 100% in DA release and reuptake were measured by utilizing microdialysis in vivo. In vitro studies were performed using Nafion-coated carbon fiber electrodes. DA release was stimulated by a 300pA of 120mM KC1 current ejected from a microelectrode attached to the recording electrode, at the 0.5 mm intervals through the dorsal-ventral and medial-lateral extents of the midbrain. Mice receiving GM1 and clearance time were determined at each location. In MPTP/saline-treated mice, the greatest decrease in DA release increased in the lateral striatum (39% of normal) and GM1 treatment increased release by almost 25%. The clearance time for released DA increased the most in the dorsolateral striatum in MPTP/saline-treated mice and decreased by almost 30% after GM1 treatment. These changes coincided with a 92% decrease in striatal DA, mazindol binding in GM1/saline-treated mice vs. a 67% decrease in 3H mazindol binding in GM1/saline-treated mice. These results further demonstrate the ability of GM1 ganglioside to enhance dopaminergic neurotransmission after a DA-depleting lesion. Supported by NIA grant AG 10280.

**670.17**
GM1 GANGLIOSIDE TREATMENT INCREASES DOPAMINE SYNTHESIS IN RESIDUAL NIGROSTRIATAL NEURONS IN MPTP-TREATED MICE. J.S. Schneider and L. DiStefano. Dept. of Neurology, Hahnemann University School of Medicine, Philadelphia, PA 19102.

GM1 ganglioside has been shown to increase striatal dopamine (DA) levels after various types of lesions to the nigrostrial DA system. Both in vivo and in vivo studies have suggested that GM1 ganglioside treatment may have rescue and survival effects on damaged DA neurons and may stimulate a spontaneous response in striatal DAergic terminals. The present study was conducted to assess the extent to which GM1 treatment may also cause a biochemical upregulation in residual DAergic neurons after an MPTP-induced lesion. Young (8-12 week old) C57Bl6J mice were administered MPTP (20 mg/kg, twice daily for 5 days) and then saline or GM1 ganglioside treatment. Some animals received injections of dibutylthiophosphate (DBU, 400 mg/kg) prior to MPTP injections and then received 3 weeks of saline or GM1 ganglioside treatment. Some animals were injected with NDS-1010 (100 mg/kg, 30 min. prior to sacrifice) to allow measurement of striatal dopa accumulation consequent to aromatic amino acid decarboxylase activity. MPTP/saline-treated animals had 46% less accumulated dopa than normal animals whereas MPTP/GM1-treated animals had only 17% less dopa than normal. DAergic+MPTP-treated animals had more severe dopamine depletions than MPTP-treated animals and striatal dopa levels 41% of normal. GM1 treatment had no effect on DA or dopa levels in these animals. These data suggest that under certain conditions, GM1 treatment can enhance the function of residual DA neurons by increasing dopa synthesis. Supported by NIA grant AG 10280.

**670.18**
INTRANIGRAL INFUSION OF CNTF, BUT NOT NGF, EGF OR TGF-B1, RESTORES STRIATAL DOPAC BUT NOT DOPAMINE LEVELS IN MPTP-TREATED MICE. T. W. Farris*, L. DiStefano and J. S. Schneider. Dept. of Neurology, Hahnemann University School of Medicine, Philadelphia, PA 19102.

Growth factors (GFs) may rescue or support damaged dopaminergic neurons in vivo raising hopes for their therapeutic potential in Parkinson's disease. However, the degree of tissue trauma from surgery and CNS delivery of GFs has varied greatly across experiments. Mechanical damage to the CNS can induce considerable release of GFs, complicating the interpretation of results following their administration. In this study, we have minimized non-specific tissue trauma to better isolate GF effects on recovery of DA-ergic markers following an MPTP-induced lesion. 8-week old male C57Bl/6J mice were administered MPTP (20 mg/kg, ip) or saline twice daily for 5 days. Two days later mice were implanted with 30 g. cannulae connected to low flow osmotic pumps (0.2 ul/h) for delivery of rhCNTF (20ug/14, rh-EFG (4ug/14), rh-BFGF (4ug/14), rh-TGF-B1 (2ug/14) or vehicle to the substantia nigra (SN) or striatum (ST). Infusion of CNTF into SN of MPTP-lesioned mice restored ipsilateral striatal DOPAC levels to 88.5% of vehicle-infused vehicle-infused controls (p=0.01, Dunnett's test) while DA levels were unaffected (34% of control). EGF, BFGF and TGF-B1 had no effects on striatal DA (27.4%, 33.4% and 30.7% of control, respectively) or DOPAC (34.7%, 43.7% and 39.2% of control). Striatal infusions of GFs had no effect. Similar experiments with these GFs in our lab employing more traumatic delivery methods have produced more positive results. We speculate that trophic substances released by trauma may be crucial for some GFs to alter DA-ergic markers. Supported by NIA grant AG 10280.

**671.1**

Cell death occurs by either apoptosis (programmed cell death) or necrosis. Apoptosis occurs as a result of the normal life span of differentiated cells, in tumors, and many other physiologic and pathologic conditions. Biochemically, the DNA of apoptotic cells undergoes cleavage via endonucleases, which are controlled by p53 nuclear-coded genes. These fragments can be identified in situ by the addition of labeled nucleotides with the enzyme terminal deoxynucleotidyl transferase (TdT). Several studies have investigated this technique in the study of cell death in disease processes of the CNS. We describe the application of this technique to various disease processes in the rodent and human CNS. We have investigated the use of TdT labeled DNA fragments as a method to distinguish apoptosis from necrosis in in situ brain samples. Autoradiography and in situ hybridization using radioactive labels were used to distinguish from each other, necrosis and apoptosis. Studies are underway to investigate the potential of this method to label cells undergoing cell death by necrosis. This method can be used to label dying CNS cells in a variety of disease states. However, it may be useful at this time if it identifies cell death by necrosis as a mechanism associated with cell death in the CNS, which is almost always the case of dying. Supported by NIH NS20856 and the Hereditary Disease Foundation.

**671.2**
OVEREXPRESSION OF THE S-100 GENE IN DOWN'S SYNDROME: A STUDY IN LYMPHOCYTES. H. Riezli, L. G. Gaudreault, J. Cloitre and M.R.V. Murphy. Dept. of biochemistry, Fac. of Medicine, Laval University, Quebec City, Canada. The S-100 gene which is located on the long arm of chromosome 21, is most commonly observed in astrocytes, may be implicated in the neurologic abnormalities observed in trisomy 21 which leads to Down's syndrome (DS). However, overexpression of this gene has not been consistently observed in other tissues, including lymphocytes. We have measured the level of S-100 mRNA in lymphocytes of 12 patients with DS (25 to 47 years old) and controls by performing semi-quantitative reverse transcription combined with polymerase chain reaction (PCR). The average level of S-100 mRNA was 2.3 fold higher in the DS group as compared to the controls (p<0.001). However, two DS individuals were clearly distinguishable. The first (n=5) did not overexpress S-100 mRNA, while the second (n=7) overexpressed the gene by 2.4 to 5.5 fold (p=0.001) when compared to age-matched controls. A negative correlation of the S-100 mRNA level with age was found in normal individuals (r=0.60; p<0.005). The DS individuals of the first subgroup showed similar negative correlation with age in their S-100 mRNA expression (r=-0.60; p<0.005), but in the second subgroup, there was no significant correlation of the S-100 mRNA level with age. Using genomic DNA amplification of the S-100 gene in 5 DS patients and 5 non-DS patients, we have shown that the DS patients were in fact trisomic for this gene. Therefore, these data suggest that a proportion of DS patients examined a S-100 gene as do normal individuals whereas another proportion overexpress this gene in a manner which is higher than the 1.5 fold expected. The degree of mental deficiency of the DS individuals, based on their learning capabilities, was estimated in a blind study. This was found to be negatively correlated with the S-100 gene expression. These data suggest that S-100 expression may be implicated in controlling the severity of the mental deficiency in adults with DS.
671.3

TAU PATIENTS IN NEURODEGENERATIVE DISORDERS: BIOCHEMICAL ANALYSIS. Y. Bule-Scherrer, L. Bule, P. Veremich*; P.R. Hof, L. Leventhal, L. Collins, G.B. Hof, U156, Lille, France; Montauk Medical Center, New York, USA.

Neurofibrillary tangles (NFT) are found in a number of neurodegenerative disorders including Alzheimer's disease (AD), progressive supranuclear palsy (PSP), amyotrophic lateral sclerosis/parkinsonism-dementia of Guam (ALS/PDC), post-encephalitic parkinsonism (PEP), and in the hippocampal formation of elderly non-demented individuals. NFT result from the aggregation of abnormally phosphorylated tau proteins into paired helical filaments or straight filaments. In the brain of patients with PDC and a subset of patients with AD, use of Western blotting and antibodies directed against phosphorylated and non-phosphorylated tau proteins, to investigate whether NFTs could be distinguished by differences in tau profile. Using specific antibodies to abnormally phosphorylated tau proteins, a triplet of proteins referred to as tau 55, 64 and 69 (or 68), was consistently detected in brain homogenates from AD cases and D5 patients. In the AD cases, this triplet was never found in any brain regions of non-demented elderly individuals, with the exception of the hippocampal formation. In PSP brains, a doublet of abnormally phosphorylated tau proteins (tau 64 and 69) was systematically found in both cortical and subcortical regions. In ALS/PDC cases, the characteristic tau triplet was found in both cortical and subcortical areas. In all PEP cases, a triplet of tau proteins was also found, but their isocortical distribution was quantitatively different among cases. As expected, in Huntington disease (HD) cases younger than 60 years, abnormal tau proteins were not found since no NFT are observed. In all of these neurodegenerative disorders, a very close relationship was observed between the tau pathology in the association cortical areas and intellectual impairment. With this approach, we were able to distinguish at least two types of neurodegenerative disorders: the Alzheimer type (AD, AD, ALS/PDC, PEP) and the PSP type.

This work is supported by the French Research Ministry, the France Alzheimer Association, NIH Grants AG05138, AG08802, and the Brookdale Foundation.

671.5

NEURONAL DEGENERATION IN THE PRIMATE DORSAL LATERAL GENICULATE NUCLEUS (LGN) FOLLOWING ELEVATION OF INTRAOCULAR PRESSURE. A.J. Hurtig, P.L. Kaufman, W.C. Huntington, The Waisman Center*, Department of Ophthalmology and Visual Sciences**, Department of Comparative Biosciences, and Wisconsin Regional Primate Research Center*, University of Wisconsin, Madison, WI.

Elevation of intracocular pressure (IOP) is considered to be an important causative factor associated with glaucoma, a leading cause of blindness. Using intracarital sodium nitroprusside, we have described previously the degenerative effects that increased IOP has on single ganglion cells in the primate retina. (Invest. Ophth. and Vis. Sci. suppl. "94). Here we describe the changes that elevated IOP has on neurons in the LGN, the primary target of retinal ganglion cell axons. IOP was elevated experimentally in one eye of five rhesus monkeys by focal scarification of the trabecular meshwork using an agen laser. Two weeks after elevation of IOP, the eyes were enucleated and immersion fixed. Sections were cut at 50 microns and stained with synaptophysin. In the LGN, the changes observed were a decrease in the size of the LGN, a decrease in the number of synapses per ganglion cell, and an increase in the number of dendritic spines per ganglion cell. With this animal model, we have shown that glaucomatous optic neuropathy is associated with degenerative changes in the LGN.

671.6


Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant disorder characterized by progressive degeneration of the cerebellar cortex and brainstem. The mutation causing the disease is an expansion of a trinucleotide repeat (CAG) which size correlates with the severity and age of onset of the symptoms. Recent cloning and characterization of the SCA1 gene revealed that the transcript is 10,606bp and that the CAG repeat resides within a coding region of 2484bp and codes for a glutamate tract. Sequence analysis did not reveal any homology with known proteins and it was found that the SCA1 gene product is a large protein, 1194amino acids, with an estimated molecular weight of 87Kd. To begin to understand the biology of the SCA1 gene, we initiated studies aimed at characterizing the expression patterns of both the transcript and the encoded protein. To this end, we have performed Northern blot analysis using mRNA from different human tissues revealed that mRNA transcribed in all tissues examined. Analysis of SCA1 expression in adult mouse brain by use of in situ hybridization revealed widespread expression throughout the central nervous system. Regions of enhanced SCA1 expression include the cerebellum (Parvinke and granule cells, as well as neurons within the deep cerebellar nuclei) and thalamus. Analysis of RNA expression from normal and SCA1 animals revealed that the allele with the expanded CAG repeat is transcribed. This argues against a pathogenetic mechanism involving loss of function at least at the RNA level. Polyclonal antibodies have been generated using either synthetic peptides or a fusion protein as an immunogen. Western blotting analysis revealed that the protein expression pattern is similar to that of the RNA. Detailed characterization of the antibodies is currently in progress to confirm their specificity.

671.7

APOLIPOPROTEIN (APO) ISOMERS IN PATIENTS WITH LEWY BODIES (LB). M.G. Martinoni*, J.Q. Trojanowski*, V.-M.Y. Lee*, M.L. Schmidt*, E.J. Hurtig*, J.P. Julius*, and C. Clark*, 1; Centre for Research in Neuroscience, McGill Univ., Montreal General Hospital, Montreal, Que., H3C 1A4, Canada, Department of Pathology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104, USA.

Lewy bodies (LB) are small round eosinophilic inclusions found mainly in brainstem nuclei and cerebral cortex. The presence of LB in the pigmented neurons of the substantia nigra is a hallmark of idiopathic Parkinson's disease. The presence of cortical LB and dementia is often called diffuse Lewy body disease (DLBD). When sufficient plaques and tangles are also present the condition may be called Lewy body variant of Alzheimer's disease (LBVAD). Lewy bodies are seen in about 30% of patients with Alzheimer's disease (AD). Apolipoprotein E (APOE) is a lipoprotein expressed in liver and brain as one of 3 isomers (APOE 2, APOE 3 and APOE 4). Recent findings suggest that the presence of APOE 4 with an increased risk for late onset AD. We examined the frequency of APOE isomers in a cohort of patients with brainstem LB, some of whom had cortical LB with or without coexisting plaques and tangles. The presence of APOE 4 was determined by PCR techniques using DNA isolated from frozen cerebellar cortex. Anonymously donated brain samples were used for the control group genotype. Our preliminary results show that while there is a slight increase in the frequency of APOE 4 in subjects with LB, the correlation is strongest for those patients with co-existing plaques and tangles.

671.8


The pathophysiological basis of neuroleptic-induced tardive dyskinesia (TD) remains unclear. The dopamine supersensitivity hypothesis can not account for the time course or the onset of TD nor the persistence of TD and the associated structural changes after neuroleptics are discontinued. Neuroleptics can induce a vicious cycle of cellular processes that lead to a cascade of oxidative stress and glutamatic activation in animals. Haloperidol increases glutamate (Glu) concentration in the striatum and Glu agonists can enhance the activity of late onset of free radicals. Free radicals on the other hand, causes release of Glu from rat hippocampal slices. Conventional neuroleptics have recently been shown to inhibit oxidative stress by 1 of the mitochondrial electron transport chain. Failure of the electron transport chain causes the formation of free radicals and may make neurons more vulnerable to excitotoxicity. We have studied 13 patients with TD and 13 patients on neuroleptics without TD to determine whether the oxidative stress is related to change in glutamatic transmission in human TD. Our data support the hypothesis that oxidative stress and glutamatic transmission. TD patients have lower superoxide dismutase (SOD) activities (p<0.05) and higher Glu, Aspartate, and N-acetylaspartylglutamate concentrations (p<0.01) in their CSF compared to non-TD patients. There is a significant correlation between SOD and aspartate, SOD and glycine. It is plausible that the pathophysiology of TD can be better explained by the interaction between oxidative stress and glutamatic transmission.

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671.0 PROTECTION OF CELL INJURY AGAINST OXIDATIVE STRESS BY RESVERATROL. S.J. Lee, Y. Cheng and A.Y. Sun*, Dept. of Pharmacology, Univ. of Missouri, Columbia, MO 65211.

There is a general consensus that free radical generation leads to alteration of biochemical and biophysical properties of cell membranes and in turn, these changes may lead to pathological manifestations in aging and other neurodegenerative diseases. Recent epidemiological studies have indicated an inverse relationship between moderate consumption of wine and incidence of coronary heart disease. This "French paradox" has drawn considerable attention and analysis has identified the presence of trans-resveratrol (R) in wine. The antioxidant properties of R may contribute greatly to protection of the endothelial cells lining the arterial walls from oxidative damage. We have investigated the possible protective effect of R in the nervous system after various oxidative insults. PC12 cells were used because of their resemblance to catecholamine neurons. An increase in cell death, as indicated by the extent of intracellular LDH released to the incubation medium, was observed after PC12 cells were exposed to F2-*/DETPAC (0.1 mM) for 36 hrs. R was able to exert a dose-dependent protective effect on the F2-*/DETPAC-induced cell death. Furthermore, the combined action of R plus Vitamins C and E was far better than the use of each individual antioxidant alone. The result indicated that R may be a potential therapeutic agent for the prevention and treatment of neurodegenerative disease (Supported in part by NIH Grant AA02054).


Earlier studies have shown that PHMK-801 binding to the NMDA receptor ion channel is decreased in spinal cord tissue from ALS patients compared to controls. This reduction could be completely reversed by the activation of protein kinase C by phorbol ester. Later work has suggested that these studies indicated the potential that NMDA receptors in spinal cord tissue may be altered by abnormal regulation of enzyme activity. PHMK-801 binding was increased after exposure to PDB (15 µM) in both control and ALS spinal cord sections. In the present study, a return of these media resulted in a further enhancement of binding in specific PHMK-801 binding to the original levels with time, where t½ = 60 min. for control and 15 min. for ALS. The rate constants for PDB-induced effects on PHMK-801 in control and ALS were 0.01/min. and 0.05/min., respectively. In control tissue the decay to the original binding level could be blocked by phosphate blockers, NAV (sodium orthovanadate), a tyrselective inhibitor) and NABD (Sodium D-3-glycerophosphate, a ser/threonine inhibitor). For NABD, half maximal block was achieved at the concentration of 10-7 M for control and 10-6 M for ALS. Almost complete block (80%) was achieved at 10-6 M for control whereas in ALS a complete block could not be achieved. These differences between ALS and control spinal cord may indicate fundamental alterations in the activity, amount or type of endogenous phosphatase in spinal tissue in ALS patients.


FACI is a developmentally regulated protein that is localized throughout the cell cytoplasm during human brain development but found primarily in the nucleus after birth. We have isolated a 160 kd FACI protein from fetal brain by affinity chromatography and report that FACI interacts with the 160 kd neurofilament protein (NFP-M). Other cytoskeletal elements such as NFP-L, tubulin, and MAPs do not co-elute with FACI. Therefore FACI represents a novel neurofilament associated protein. Neurofilament abnormalities are present in many neurologic diseases such as Alzheimer's disease (AD), Friedreich's ataxia, and amyotrophic lateral sclerosis (ALS). FACI abnormalities have also been found in these diseases. Within the neocortex of AD brain FACI is present in a subset of neuritic plaques (Alzheimer's disease) and in non-neuritic plaques in the amygdala and ALS spinal cord by immunoelectronmicroscopy. In the developing lumbar cord FACI resides in nuclei of multiple cell types, with highest protein levels in anterior cord and lowest FACI in posterior cord. FACI is detectable in cells with symptoms of ALS, including motor neurons. FACI is present in motor neurons and in axon tracts. We are currently determining the expression of FACI in cells with NF abnormalities or if FACI expression precedes NF changes. Results of in situ hybridization studies using probes for FACI mRNA will also be presented. Re-expression of FACI may be characteristic of cell responding to early cellular abnormalities that occur during AD and ALS.
671.15

AMYOTROPHIC LATERAL SCLEROSIS IMMUNOglobulINS INCREASE INTRACELLULAR CALCIUM IN A MOTEURONE CELL LINe, L.Y. Colom*, M.E. Alexiou, R.G. Smith and S.H. Appel, Department of Neurology, University of Washington, Seattle, WA.

Amyotrophic Lateral Sclerosis (ALS) is a devastating motoneuron disease of unknown cause. Recent evidence suggests that autoimmune mechanisms are involved in the origin and progression of the disease. We have previously demonstrated the presence of antibodies to voltage-dependent calcium channels in ALS patient sera, and shown that ALS IgG induces calcium-dependent cytotoxicity in an hybrid motoneuron cell line, VSC4.1 (Smith et al., 1993:339-97, 1994).

These ALS IgG also increase the amplitude of voltage-dependent calcium currents in VSC4.1 cells (Mouler et al., Soc. Neurosci. Abstr: 1996: 3). To determine whether ALS IgG can increase intracellular calcium in VSC4.1 cells, we have employed confocal scanning microscopy and the calcium-sensitive dye Fluo-3. In VSC4.1 cells, basal intracellular calcium levels were 74±32 mM. Increases in intracellular calcium were observed in 29% of cells, following bath addition of IgG from 6 ALS patients. Two types of intracellular calcium changes were observed: 1) an early fast transient (364±233 mM), appearing 15-120 sec after adding ALs IgG to the bath, and 2) a slower, progressive increase appearing 30-120 min later. Of the cells that showed a fast transient, 1/3 developed a slower progressive calcium increase (compared to less than 10% of the cells that lacked a fast transient). Addition of IgG from 4 control patients produced calcium increase in only 1% of cells. ALS IgG failed to produce fluorescence changes in calcium free media. These findings suggest that ALS IgG induces an early calcium influx through the cell membrane which, despite its short duration, may initiate a cascade of events leading to a later progressive increase in calcium and subsequent cell death. This work was supported by M.D.A. and Cephalon, Inc.

671.16


Among the Chamorro people of Guam, the high incidence and prevalence of ALS has decreased in the past decade, while parkinsonism-dementia complex (PDC) continues to be frequently encountered. We have recently noted the frequent occurrence of cases of dementia who remain free of amyotrophy or parkinsonian signs through the entire course of their illness. Pending further characterization of this disorder, we have referred to such cases as Marianas dementia. We now report neuropathologic findings on 7 pure dementia cases (73 ± 8.5 years old, duration of illness 3.5 ± 1.6 years) among inhabitants of Guam. None of these cases showed evidence of amyotrophy or extrapyramidal features despite careful neurologic surveillance. Five of these cases were of Chamorro extraction and had been life-long inhabitants of Guam. These cases showed evidence of severe cerebral atrophy with widespread neurofibrillary tangles (NFT), in the absence of neuritic plaque formation. The NFT heavily involved limbic structures and predominated in layers III/IV (as opposed to layer V) of the neocortex. There was loss of neurons in the substantia nigra with NFT in the upper brainstem. These 5 Chamorro cases were virtually indistinguishable neuropathologically from PDC. The remaining 2 cases were migrants to Guam (82 year old Caucasian, 83 year old Filipino) and both showed typical neuropathologic features of Alzheimer’s disease. This study demonstrates that a purely dementing syndrome, in the absence of amyotrophic or parkinsonian manifestations represents an additional form of ALS/PDC among at-risk Chamorro natives of Guam with chronic exposure to unknown environmental factors present on the island.

671.17


Cycasin, the 6-D-glucose of methylazoxymethanol (MAM), is a possible ecological factor for western Pacific amyotrophic lateral sclerosis/Parkinsonism dementia complex. We have hypothesized that these genotoxins act as slow toxins by permanently altering post-miotic neural tissue DNA. In previous studies, we demonstrated that MAM-induced neurotoxicity in vitro is potentiated by O^-hcytrazygmine (O^-Bjg), an inhibitor of the DNA-repair protein methylazoxymethanol/N-methyl-N-hydroxyethylpyruvate (MGMT). To further examine the relationship between DNA damage and MAM-induced neurotoxicity, MGMT levels and DNA damage were determined in mouse cortical explant tissue, primary rat cortical astrocytes, and primary rat cerebellar granule cell cultures treated for 1-3 days in the presence or absence of 5.0 µg-O^-Bjg/mL 0.1 mM of the genotoxins MAM, N-methylazoxymethanol, or procarbazone. Western blots demonstrated MGMT in protein extracts of all three tissue types, with particularly low levels in granule cell cultures. MGMT levels were reduced in explants and astrocytes treated with O^-Bjg. The level of peroxidation was increased in explants from explants treated with O^-Bjg. DNA damage, as determined by immuno-dot-Blot using monoclonal antibody EM-21 to the DNA adduct O^-methyl-N-hydroxyethylpyruvate (O^-MeAO), was not detected in cultures treated for 24 h with MAM or other genotoxins. However, O^-MeAO was detected in all cultures treated for 3 days with MAM. DNA damage levels increased 2-3 times when explants were treated with MAM and 5.0 µg-O^-Bjg. Taken together, the present studies indicate that modulation of DNA repair can increase the susceptibility of nervous tissue DNA to damage by genotoxic agents that may lead ultimately to cell death. [Supported by a grant from the Medical Research Foundation of Oregon and NS19611]

NEUROTOXICITY: DRUGS

672.1

The Organophosphate Insecticide - Chlorpyrifos: Precovalent & Behavioral Effects in Adult Rats. John N.D. Watan* and Jesse H. Elmadjian, Department of Pharmaceutical Sciences, College of Pharmacy, St. John's University, Jamaica, NY 11432.

Chlorpyrifos (CP) has been shown to have proconvulsant effects in immature rats (Hupfer et al. 1993) and elicits behavioral effects when administered pre- or post-natally (Muto et al. 1992). These early studies also demonstrated an interaction of CPF and xyl (XYL). Experiments were performed to determine both the effects of CPF on kindling and behavioral neurotoxicity in adult rats. Xyl was administered alone or in combination with CPF. Rats (Taconic Farms) weighing 175±25 grams received amygdalar electrodes 1 week prior to initiation of kindling procedures. CPF or XYL, alone or in combination, was administered subcutaneously in 10% peanut oil/vitamin E (VHE). Dose range: CPF 0.3-100 mg/kg; XYL 0.2, 0.5 or 1.0% in all cases N=2. The afterdischarge threshold (ADT) was determined in rats treated with CPF, XYL, of XYL/VHE. No differences were noted between the groups. ADT was not affected by treatment. Kindling was performed (1 stimulus, 100 µA, 1 sec duration, 60 Hz, square wave pulses) in CPF, XYL, XYL+CPF or VHE treated rats. All XYL + CPF and CPF treated rats showed a decrease in kindled response to its absence. Kindling was performed (1 stimulus, 400 µA, 1 sec duration, 60 Hz, square wave pulses) in CPF, XYL, XYL+CPF or VEH treated rats. CPF and XYL treatment displayed additively (kindling rate accelerated). CPF displayed neurotoxicity in adult rats as determined by the rotarod test. XYL appeared to be without effect in this test. The combination of CPF and XYL displayed additively in this test. Spontaneous Motor Activity (SMA) displayed a biphasic effect of CPF; at 2 hours post-CPF activity increased modestly in a dose-dependent manner. At 36 hours CPF elicited a modest decrease in SMA. Highest doses used XYL caused an increase in SMA at 2 hours, yet at 36 hours SMA did not differ from VEH-treated controls. CPF + XYL demonstrated additivity at 2 hours post dose. Brain cholinesterase activity was determined by a spectrophotometric method. CPF caused reductions in cholinesterase activity in a dose-dependent manner from 2 hours to 72 hours following CPF administration. Studies supported by St. John's University, Col. Pharmacy.

672.2

HYDROGEN PEROXIDE HYPERPOLARIZES RAT HIPPOCAMPAL PYRAMIDAL NEURONS BY OPENING K+ CHANNELS. Y. Seunit*, J. Scuvé-Moreau, L. Massotte, A. Dresse, Lab. de Pharmacologie, Univ. of Liège (B-4000), Belgium.

Hydrogen peroxide (H2O2) may be involved in a number of pathological conditions affecting neural cells, but its electrophysiological effects have not yet been studied in detail in the rat. Using intracellular recordings of presumed CA1 pyramidal neurons in the slice preparation, we observed that H2O2 (0.3-3 mM) induces in these cells a reversible and reproducible hyperpolarization (h<0.5 mV, Mean ± SEM, N=13, by 3 mM) from resting potential (-65 ± 2 mV) and turnover that was not due to a K+ channel opening because (1) in 2.5 mM K+, it had the same reversal potential (-97 ± 2 mV, N=3) as the one of back of (10 µM) Ni2+ (± 1 mV), a known K+ channel opener; (2) its reversal potential shifted according to the Nernst equation for K+ when [K+]o was changed (from -96 ± 3 mV in 2.5 mM to -63 ± 2 mV in 10.5 mM, N=3); (3) it was reduced by a K+ channel blocker (100 µM Ba2+, N=4).

The precise identity of the channels that are affected, as well as the mechanism by which they are opened, remain to be determined.
NEUROTOXICITY: DRUGS

672.3

NEUROTOXICITY OF HYDROGEN PEROXIDE. P.A. Roantree, O. Bon, Yosef Y., N. Dujovny, B.D. Rors. Neuroscience Pharmacology, Park-Davis Research, Div. of Warner-Lambert Co., Ann Arbor, MI 48105 and Dept. of Radiology and Neurological Surgery, Univ. of Michigan, Ann Arbor, MI 48109. Free radical induced neuronal injury has been implicated in a number of neurological disorders and has been linked to excitotoxic cell death. The current experiments evaluated the toxicity produced by the direct application of hydroxyl radicals, hydrogen peroxide (H$_2$O$_2$). Experiments were performed on 17-day old cerebrocortical primary cultures under conditions similar to those employed in excitotoxic cell death. Cell death was assessed using a histologically and quantitatively by the measurement of lactate dehydrogenase (LDH) released into the medium. Following a 30 min exposure to H$_2$O$_2$, there was a concentration dependent cell death assessed at 2 hr and 24 hr (EC_{50}=100 mM). Shorter exposure periods to H$_2$O$_2$ were less effective. LDH release did not occur until > 3 hrs after removal of H$_2$O$_2$ indicating a delayed cell death, similar to that seen following exposure to glutamate. Pretreatment with the iron chelator deferoxamine (2 mM) produced a rightward shift in the H$_2$O$_2$-induced toxicity curve (EC_{50}=458 uM), while inhibition of catalase with aminotriazole (30 mM) enhanced the H$_2$O$_2$ induced toxicity (EC_{50}=54 uM). This neurotoxicity was not mediated by NMDA receptors, since the NMDA antagonist MK-801 did not prevent the cell death. These results are consistent with the assumption that the generation of hydroxyl radicals is necessary for cell death. As with excitotoxicity, a rise in [Ca$^{2+}$], about 40 min following continual exposure to H$_2$O$_2$, Removal of extracellular Ca$^{2+}$ prevented the increase in [Ca$^{2+}$], suggesting that the increase was not the result of the liberation of intracellular stores of calcium. These data demonstrate that H$_2$O$_2$-induced toxicity has many of the same features as excitotoxic cell death, suggesting that there may be a causal link between these forms of neurotoxicity.

672.5

POLYCHLORINATED BIPHENYLS REDUCE TYROSINE HYDROXYLASE ACTIVITY IN RAT BRAIN. R.F. Segal and V. Shaian, Wadsworth Center, NYSDOH, Albany, NY 12201. Polychlorinated biphenyls (PCBs) reduce dopamine (DA) concentrations in adult rat and human primates brain and in schizophrenic/hypermotoric (PC12) cells. Using neostriatal (NIE-115) cells that do not express the enzyme dopa decarboxylase (DDC), required for the conversion of L-Dopa to DA, we have shown that PCBs reduce media Dopa concentrations, suggesting that PCBs reduce the activity of tyrosine hydroxylase (TH) to convert tyrosine to L-Dopa. To determine if reductions in brain DA concentrations are also due to inhibition of TH we exposed adult Wistar-derived rats to either Acorus 1254 (1,000 ppm in chow) or control chow for 46 days and measured their 30 min after they received intraperitoneal injections of either 100 mg/kg of 3-hydroxybenzylhydrazine (NSD-1015), an inhibitor of DDC or saline. DA, L-Dopa, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations were determined in the striatum and nucleus accumbens by HPLC. PCBs significantly reduced DA concentrations in the striatum and DOPAC and HVA concentrations in both brain regions. Co-treatment with NSD-1015 resulted in further decreases in DOPAC and HVA as well as reductions in DOPAC concentrations compared to control/NSD-1015 treated animals. These reductions in L-Dopa and DA metabolite concentrations strongly suggest that PCBs, in vivo, inhibit production of newly synthesized DA at the level of tyrosine hydroxylase. Supported by NIH Grant #ES0491304.

672.6

NEUROTOXICITY OF IBOGAYNE IN CD MICE. S.F. All, M. Meneg and J.P. O'Callagham, Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR 72079, Neurotoxicology Division, US EPA, Research Triangle Park, NC 27710. Iboigayne is an indolealkylamine known to have CNS stimulating and hallucinogenic properties in animals. Recently, ibogayne has been proposed for use as a treatment for drug addiction. The neurochemical basis for such therapy, however, remains unclear. The present study was designed to evaluate the neurotoxic effects of ibogayne in mice. Iboigayne was administered to adult female CD-mice (20 g) once daily for 24 hr. On day six, animals were sacrificed and brains removed, and were stored in buffered formalin for 1 week. Sections were cut at 5 microns and stained with cresyl violet. Iboigayne produced a dose-related decrease of 5-HT in caudate nucleus (CN), frontal cortex (FC) and brain stem (BS). Iboigayne produced a significant increase in 5-HT in hippocampus (HIP). There were no significant changes in CN, however, DOPAC was elevated in CN and FC. Iboigayne increased GFAP in olfactory bulb, hippop and BS. These data suggest that ibogayne has a complex neurotroph profile in female mice, as assessed by changes in monoamine concentrations and astroglia.

672.7

CHRONIC EXPOSURE TO IBOGAINE RESULTS IN SEX-DEPENDENT ASTROGLIALIZATION. J.R. Barta, J.P. O'Callagham, L.E. Redman, T. Rogers, J.B. Trevill and J.G. Pegg. U.S. Environmental Protection Agency, Res. TII. PK, NC, 27711; Southern Res. Inst., Birmingham, AL 35232; NIDA, Rockville, MD 20857. Iboigayne (IBG) is a naturally occurring alkaloid isolated from the root of the African shrub, Tabernanthe iboga. Evidence exists showing that IBG interrupts the physiological and psychological aspects of drug addiction, both in experimental animals and in the clinic. However, the mode of action of IBG to cause neurotoxicity in male and female rats as evidenced by the presence of astrogliosis, a generic response to CNS injury. IBG was administered to male and female Sprague-Dawley rats (5, 25, 50, or 150 mg/kg, p.o) once daily for 14 days. Rats were sacrificed on day 15 and 31. Brains were dissected into olfactory bulb, hippocampus, striatum, cortex, brainstem and cerebellum. IBG-induced astroglia was quantified by assaying the content of the astrocyte intermediate filament protein, GFAP, in each brain region, using a sandwich ELISA. In male rats, GFAP was not elevated as a function of IBG treatment at any dose or in any region on day 15. However, female rats, exposed at 150 mg/kg of GFAP, were observed on day 15. The greatest increases were seen in olfactory bulb and brainstem, notably, the cerebellum was not affected. In both male and female rats GFAP was unaffected on day 15, following distributions of underlying brain damage. Supported by NIDA Contract 1-192 and NIDA IAG-1-Y1011-30063.

672.8

A CYTOTOXIC EFFECT OF ESTROGEN ON AMYGDALA NEURONS. K.S. Durg, J. Somba, R. Matsuyoshi, M. Kanazawa, A. Ando, S. Chin, and L. Shimada, Division of Health Sciences, University of the Air, Chiba Japan, Division of Basic Medical Sciences, Royal Free Hospital School of Medicine, London. It has been described that estrogen exerts cytotoxic effect on β-endorphin containing neurons in the rat hypothalamus. In this paper, effects of estrogen on cultured rat amygdala neurons were studied. Primary cultures of rat fetal amygdala neurons were used for the experiment. After an acute exposure of estradiol, cells were incubated for 24 hours. Then the percent survival of cells was calculated by assaying intracellular LDH activity. Effect of estradiol on intracellular Ca$^{2+}$ concentration in the cultured neurons were also examined by fura-2 fluorescence. As results, estradiol had a cytoprotective effect on amygdala neurons at L-hormone concentrations, but at M- and cytotoxic effect at higher concentrations. On the other hand, estradiol caused long-lasting Ca$^{2+}$ elevation within cultured amygdala neurons. Mechanisms of this Ca$^{2+}$ elevation was analyzed by adding dipyridamole, a Na-cytotoxin and mRNA inhibitors. Currently, in situ hybridization experiments of hepatic growth factor on rat amygdala before and after estrogen administrations are in progress.
NEUROTOXICITY:

IC50 values were measured in cerebral and forebrain using HPLC. Densities of
NMDA, kainate, adenosine-A1 and GABA-A receptors were also measured by microdialysis. By day 3, concentrations of the excitatory amino acid transmitters, glutamate and aspartate began to fall and remain low (-55% to 70%). Concentrations of GABA and glutamine increased transiently but fell back to control levels by day 5. Concentrations of taurine fell by day 3 (-60% of controls) and remained low. Glycine concentrations increased at day 3 (+200% of control values) and remained elevated. The densities of the glutamate receptors, NMDA and kainate were reduced in cerebral cortex from CPA treated rats. There was no change in the densities of NMDA or kainate receptors in any other brain region examined. Densities of adenosine A1 and GABA-A receptors in cerebral and forebrain regions were not changed following CPA administration. In conclusion, we suggest that reduced glutamate and aspartate levels and NMDA and kainate receptor densities in the cerebral cortex are related to loss in cerebral granule cells. Changes in other amino acids may reflect compensatory events in response to the toxic insult.

GLIAL FIBRILLARY ACIDIC PROTEIN IN THE RAT BRAIN RESPONSES BIPHASICALLY TO INHALED TOULENE. AR. LITTLE, ZL GONG, HAN EF-WA AT, HE Evans. Institute of Environmental Medicine, New York University Medical Center, Tuxedo, NY, 10987

GAP concentration in the hippocampus and cerebellum was evaluated as a biomarker of the neurotoxicity of inhaled toluene. Fisher-344 rats were exposed at 1000ppm or 10000ppm by inhalation for 6 hours/day, 5 days/week. Days 1, 3, 7, 21, and 42 were evaluated. GAP response in the hippocampus was biphasic at both doses, initially decreasing but later increasing above control by day 21 to day 42. In the cerebellum GAP levels declined significantly by day 3, increasing thereafter, never quite returning to control levels. These data are consistent with a hypothesis emerging from this laboratory concerning the mechanism of toluene toxicity, that low doses of toxic chemicals affect regulation of GAP. The most familiar finding is an increase in GAP typical of reactive gliosis. A less frequent and unexpected change may involve decreased GAP levels, observed with very low level exposures. Our studies also indicate a biphasic response with exposure to Pb (Gong et al., 1994). Relative low level exposures administered chronically may be necessary to observe the early phase of the gliotic response, a transient decrease followed by an increase if given enough time or a large enough dose. Low level or short term exposure to toluene may influence changes in glial cell function that precede overt toxicity characterized by gliosis Supported by the American Petroleum Institute and grant ES-04895.


Direct injection (1 µl) of the peptidic Substance-P (SP) antagonist [D-PRO2 D-TRP7,9] Substance-P (DPDP-TSP, 1-3 nmol) induced neurodegeneration in the dorsal hippocampus of rats. Co-injecting SP (50 nmol) reduced the toxicity induced by 3 nmol of DPDP-TSP. Another SP antagonist [Arg8, D- Trp7,9]MePh(8)SP (6-11) (10 nmol) was also neurotoxic after intrahippocampal injection. Surprisingly, the nonpeptidic SP antagonist CP-96,345 was not neurotoxic at a dose of 10 nmol. The reduced neurotoxicity of the nonpeptidic antagonist was not due to a lower affinity for the SP receptor. Concp-96,345 displaced the NK-1 agonist [H3][Sar2 Met5(OH)11]SP with higher affinity (IC50 = 0.34 µM) than DPDP-TSP (IC50 = 8.9 µM) in rat brain membranes. Binding of the peptidic and nonpeptidic antagonists at different receptor subtypes or different injection sites was not enough to explain the differential neurotoxicity observed in the present experiments.

NEUROTOXICITY: DRUGS


To investigate whether the hippocampal damage caused by thiamethoxam (TMT) involves corticosterone (CORT)-receptors, effects of a single oral dose of TMT–chloride (TMT–c) administration on plasma CORT levels on Day 0, 3, and 4 after the TMT–c and 2) response of the level at 0, 30, 60, 90 and 120 min after corticosterone-releasing factor (CRF) injection (28 µg/kg, i.v.) on Day 4 were examined. A significant increase in plasma CORT levels was observed (p<0.05) in response to CRF injection on Day 4. The enhanced CORT levels on Day 4 were shown to be almost completely suppressed at 90, 180 and 300 min after an administration of dexamethasone of 100 µg/kg (s.c.), indicating that the CORT-receptors both in the hypothalamus and pituitary might not be affected by the TMT–c, whereas alterations of the hippocampal CORT-receptors could not be denied. On the other hand, the animals in the same condition with thiamethoxam–chloride (TET–c) of 4 mg/kg (p.o.) did not show significant increase on Day 3 or 4, but their responses of the CORT level to the CRF on Day 4 were exaggerated compared to those among the vehicle (corn oil) controls, suggesting that actions of TET–c, or the hippocampus–HPA axis may be different from those by TMT–c and TET–c.
NEUROTOXICITY: DRUGS

672.15

NEUROTOXIC EFFECTS OF MONOAMINE OXIDASE-B INHIBITORS ON DSP-4 INDUCED DEGENERATION OF NORADRENERGIC AXONS IN THE RAT BRAIN. X. Zhang, P.H. Yu and A.A. Boulton*. Neuropsychiatry Research Unit, University of Saskatchewan, Saskatoon, Canada, S7N OWO.

DSP-4 [N-ethyl-N-nitro-2-naphthylamine] is a highly selective neurotoxin toward the locus coeruleus noradrenergic (NA) system. Previous biochemical studies have shown that the monoamine oxidase-B (MAO-B) inhibitory effect of DSP-4 (R)-deprenyl and Q-210 (N,N,N-trimethylpropargylamine), are able to prevent DSP-4 induced NA depletion in the mouse hippocampus. It is, however, unknown whether this represents a neuroprotection of NA axons. Employing dopamine-β-hydroxylase immunohistochemical and imaging analysis methods, we have found that 92% and 84% respectively NA nerve fibers in the mouse hippocampus are spared from DSP-4 neurotoxicity by a single pretreatment dose of R(-)-deprenyl or 2-HxMP. Similar neuroprotective effects are also observed in the cerebral cortex, thalamus, amygdaloid complex and cerebellum. This is the first morphological evidence demonstrating that deprenyl and 2-HxMP can indeed protect NA axons against DSP-4 neurotoxicity. We are currently also investigating the neuroprotective and neurorescue effects of R(-)-deprenyl and 2-HxMP on DSP-4 induced chronic degeneration of NA neurons in the locus coeruleus.

672.17

THE EFFECT OF ACUTE ETHANOL TREATMENT ON CALCIUM HOMEOSTASIS IN CULTURED HIPPOCAMPAL NEURONS BEFORE AND DURING DEPOLARIZATION. B.Webb*, S.S. Suarez, M.D. Heaton, and D.W. Walker, University of Florida, V.A. Medical Center, Gainesville, FL 32601.

The neurotoxic effect of acute ethanol treatment (AET) may lead to an alteration in the regulation of calcium homeostasis in hippocampal neurons. We cultured E18 rat hippocampal neurons for 2 weeks. The cultures were loaded with 2μM indo-1/AM for 1 h at 37°C, rinsed with buffer, and maintained for 1/2 h at 32°C. The cultures were divided into four groups: control, 100,400,800 μg/ml ethanol. Control readings were taken, and then the buffer was changed for either control buffer or buffer containing 100,400,800 μg/ml ethanol. Fast responses were recorded for the first 6.4 sec and then at 10,30,45,60 min. To determine the effect of AET on calcium homeostasis in cultured hippocampal neurons during depolarization, the buffer was changed after 60 min to either control buffer and 30mM KCl or buffer with ethanol and 30mM KCl. Fast responses were recorded for the first 3.2 sec and then at 5.1 min. In cultures treated with 400 or 800 μg/ml ethanol, [Ca2+]r was increased (p<0.01) at later time points, 400 and 800 μg/ml treatment decreased [Ca2+]r in a dose-dependent manner. When AET cultures were compared to control, both 100 and 400 μg/ml ethanol increased resting [Ca2+]r (p<0.01) at 10 min (p<0.05). At later time points, 400 and 800 μg/ml treatment decreased [Ca2+]r in a dose-dependent manner. The results of this study indicate that AET results in the alteration of calcium homeostasis in cultured hippocampal neurons at membrane potentials and during depolarization. These changes in [Ca2+]r may disturb normal cellular function and contribute to cell death. Therefore, the alteration of calcium homeostasis may be an underlying mechanism of ethanol neurotoxicity. Supported by NIAAA grants AA00200, AA09128, and Fellowship #1F31AA05722-01; and Medical Research Service Department of Veterans Affairs.

672.18

ETHANOL DECREASES INTRACELLULAR MYO-INOSITOL IN C. CELLS. K.J. Isenberg*, S.G. Holstled, B.W. Moore and W.B. Sherman, Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

Ethanol produces a dose-dependent decrease in the growth of C6 glioma cells. We hypothesized that the etiology of this phenomenon could be similar to effects observed when the cells are grown in hyperosmolar sodium chloride (NaCl). C6 cells were grown in Dulbecco's Modified Eagles Medium and 10% fetal calf serum (controls) or grown in the same media supplemented with ethanol or NaCl in chronic exposure (7 days) or acute exposure (2 days). The results of 2 day experiments reveal that ethanol has a more potent inhibitory effect on growth than equimolar additional NaCl. Unlike exposure to hyperosmolar NaCl, chronic ethanol exposure does not alter intracellular concentrations of protein. Acute exposure of C6 cells to ethanol is associated with a 50% decrease in intracellular concentrations of myo-inositol, the opposite of effects observed upon exposure of the cells to hyperosmolar NaCl. The effects of ethanol upon intracellular concentrations of myo-inositol occur in a dose dependent fashion at concentrations of ethanol (8.5 mM to 130 mM) commonly encountered in human intoxication. Growing the cells in media supplemented with myo-inositol then switching the cells to media with added deuterated myo-inositol and ethanol suggests that ethanol alters myo-inositol influx and efflux. The effects of ethanol on influx of myo-inositol appear to be the opposite of the effects of NaCl upon myo-inositol transport. Ethanol induced reductions in the intracellular concentration of myo-inositol may impair the growth of these cells.

NEUROTOXICITY: METALS

673.1

SUBCHRONIC METHYLMERCURY (MeHg) EXPOSURE ALTERS Ca"+ RELEASE FROM AN IP3-SENSITIVE STORE IN NG108-15 CELLS. J.E. Siros*, and W.D. Aitchison Dept. Pharm./Tox, Mich. State Univ., E. Lansing, MI 48824.

MeHg is a neurotoxic organomercurial which exhibits complex effects on intracellular ion homeostasis. Acute exposure to MeHg initially increases [Ca2+]r from an IP3-sensitive pool, increases the concentration of an endogenous non-Ca"-ion and ultimately leads to influx of Ca2+ across the plasma membrane. The present study was undertaken to determine if subchronic exposure to low μm concentrations of MeHg could also alter ion homeostasis and/or affect Ca2+ release from IP3-sensitive pools. 24 Hour Exposure of 1μM MeHg did not alter the apparent resting [Ca2+], but did cause an increase in the response to bradykinin (BK; 1μM). 2μM MeHg for 24 hr also increased the response to BK in addition to slightly increasing the apparent resting [Ca2+]. These results suggest that NG108-15 cells exposed subchronically to MeHg are able to overcome an initial loss of Ca2+ from the IP3-sensitive pool. The enhanced response to BK may reflect an ability of MeHg either to facilitate release from the IP3-sensitive pool or possibly to inhibit the uptake of Ca2+ following its release from this pool. Supported by NIH grant ES03299.

673.2

NEUROPSYCHOLOGICAL AND PSYCHIATRIC COMPLICATIONS ASSOCIATED WITH HIGH LEVELS OF SERUM COPPER OF UNKNOWN ETIOLOGY. W.J. Coughlin II, R.D. Fastie*, and J. Joyce*, Human Neurology Laboratory, The American University, Washington, DC 20066, Mt. Vernon Center for Community Mental Health, Alexandria, VA 22309, and *Psychiatric Associates of Fredericksburg, Fredericksburg, VA 22401. Chronic copper intoxication is characterized by Wilson's disease, a rare genetic disorder that usually manifests as either a hepatic or neurological syndrome. Wilson's disease is fatal unless treated with chelating agents. Death results from the direct effects of copper toxicity on the liver. Although the subjects in this investigation do not meet diagnostic criteria for Wilson's disease and are without gross symptoms, laboratory blood tests have indicated toxic levels of serum copper of unknown etiology that are as high as those typically reported in Wilson's patients. The subjects in the present study suffer from psychiatric disorders and approximately 20% of Wilson's patients initially exhibit psychiatric symptoms prior to the onset of the full clinical entity. Using a comprehensive battery of psychological tests, we assessed two female psychiatric patients with chronic copperemia. Preliminary results indicate deficits in verbal and nonverbal fluency, verbal and nonverbal recall, motor speed, and motor coordination. Comparative neuropsychological studies on Wilson's disease in the literature, to date, have found comparable deficits in verbal recall and motor speed. Explanations for the presence of hypertymbria in the subjects are explored including the possible existence of a previously unknown subtype of Wilson's disease. We suggest it is possible, in some rare instances, that patients may present with psychiatric cognitive, or motor symptoms that may, in fact, be related to undiagnosed hypercupremia.
NEUROTOXICITY:

MANGANESE (Mn) poisoning is an occupational disease whose symptoms resemble the Parkinson's. This study is aimed at assessing the effects of Mn in the "H-Spiroperidol receptor pattern of different regions in mice. Male adult mice received a daily intraperitoneal (i.p.) Mn dose of 6mg/kg for eight weeks. Control mice were injected with saline. At the end of assay, the mice were killed by cervical dislocation, and the brains were removed on ice. Olfactory bulb, striatum and hypothalamus were dissected out and frozen until the assay was performed. After that, samples of each region were taken for Mn determination. "H-Spiroperidol binding assays were realized according to the method described by Shargya. Mn concentrations were determined by atomic absorption spectrophotometry. Mn-treated mice showed a significant increase of Mn levels in all regions. The Emax and Kd values for "H-Spiroperidol binding were unaltered by the treatment. Our investigation reinforce previous findings which seem to point out that mice submitted to this conditions are in an early stage of Mn poisoning, where cell membranes damage has not occurred.

METHYL MERCURY INDUCED NEUROTOXICITY IS BLOCKED BY ANTI-OXIDANTS S.T. Park1,2, K.T. Lim1, Y.T. Chang1 and S.U. Kim1. 1Div. of Neurology, Dept. of Medicine, Univ. of British Columbia, Vancouver, BC, V6H 3L5, Canada. 2Dept. of Anatomy, Wonkwang Univ. School of Medicine, Iri 570, Korea.

Methyl mercury compounds are known to induce neurotoxic changes in the mammalian central nervous system (CNS). In order to characterize the mechanism of methyl mercury neurotoxicity, neonatal mouse cerebral neural cultures were exposed to graded concentrations of methyl mercury chloride (0.1-0.02 mg/m) for 2.48 hours. Cell viability was determined using MTT assay and neutral red uptake assay. Methyl mercury was toxic to mouse cerebral neural cultures (LD50 = 20μg) after 24 hours of exposure, and neurotoxicity was blocked in a dose-dependent manner by catalase (10-100 μg/ml) and glutathione (0.2-2 mM). Other reagents tested for neuroprotective effects against methyl mercury toxicity such as selenium (1-10 μM), ascorbic acid (20-200 μM), sodium thiosulfate (0.1-1 mM) and cytoxine (50-500 μg/ml) were not effective. These results indicate that anti-oxidants such as catalase and glutathione are effective in blocking methyl mercury neurotoxicity in the CNS.

EFFECTS OF ALUMINUM ON NEURONAL ION CHANNELS C.H. Ka and I. Saya. Dept. of Pharmacology, Northwestern University Medical School, Chicago, IL 60611-3008, and Dept. of Physiology, Hiroshima University School of Medicine, Hiroshima 734, Japan.

Aluminum accumulation in the body during long-term kidney dialysis has been implicated in the pathogenesis of seizure in dialysis encephalopathy. However, the mechanism of seizure induction is unknown. Previously, we have shown that external application of aluminum caused a dose-dependent depolarization of axon membranes, prolongation of the action potential duration, and repetitive firings of action potentials (Na, FASEB J. 7:6998, 1993). The aim of this study was to elucidate the mechanism of aluminum-induced neuronal hyperexcitability at the ion channel level. Voltage clamp experiments were conducted on giant axons of squid and crayfish. In intact axons, external application of aluminum acetylacetone (1-2 mM) caused a decrease of the potassium current without significant effects on the sodium current. The reduction of K conductance was dose-dependent, and the effect had a slow onset. In internally perfused axons, aluminum had no effects whether it was applied externally or internally. The results suggest that neuronal hyperexcitability seen in dialysis encephalopathy is due to inhibition of potassium channels by aluminum and the inhibition is probably mediated by cytoplasmic constituents. (Supported by NIH grant NS30101).

ALTERED LEVELS OF NERVE GROWTH FACTOR AND ITS LOW-AFFINITY p75 RECEPTOR IN THE DEVELOPING BRAIN FOLLOWING PRENATAL EXPOSURE TO MERCURY VAPOUR. Susan Sidderson1,2 and Ted Fereday1*. Dept. of Developmental Biology, Uppsala University, Box 587, S-751 23 Uppsala, Sweden. Pregnant rats were exposed to low levels of mercury vapour. The levels of mercury (5-10 μg/g wet weight) found postnatally in the brains of their offspring are comparable with those found in human brains after average mercury vapour exposure. The brains of the pups show an approximately 50% decrease of NGF protein in the hippocampus, and the cortex at postnatal day 21 with a concomitant decrease of 50% of normal levels in the medial septal area whith NGF responsive cholinergic neurons. The levels of mRNA for the NGF affinity receptor p75 was significantly reduced to approximately 30% of normal in both the medial septal area and in the diagonal band nucleus. ChAT mRNA was slightly reduced in the diagonal band and the medial septum and was significantly reduced in the striatum. It is suggested that the retrograde transport of NGF from the target to the basal forebrain was interrupted due to the reduced mRNA levels. Moreover, NGF produced by the fibroblast cell line 3T3 in the presence of organic mercury (MeHgCl) was found to be doubled when methyl mercury was added at concentrations of 0.1 μM-0.5 μM.

METALLOTHIONEIN: ISOFORM PURIFICATION IN HUMAN FRONTAL CORTEX K. Kittingham E. J. Kaspris, Graduate Center for Toxicology and Department of Neurology, Veteran's Administration Medical Center and University of Kentucky, Lexington, KY 40536.

Metallothioneins (MTs) are a family of ubiquitous cysteine-rich, 6-7 kD proteins which detoxify heavy metals, participate in zinc homeostasis, and may function as an intracellular antioxidant. Six MT isoforms have been sequenced from human liver. Immunocytochemical data suggest multiple isoforms in the central nervous system; however, only one isoform, MT-III, has been sequenced from human brain. MTs were purified from cortex by homogenizing in 5 mM Tris-HCl, followed by ultracentrifugation. After heat precipitation, the soluble fraction was treated with chymotrypsin. Residual proteins and metals were removed by gel filtration. The MTs were then derivatized with 4-vinylpyridine and the 4- pyridylethylated derivatives were separated by reverse phase HPLC. Sequencing data suggest the presence of multiple isoforms in the cortex in addition to MT-III. Alterations in the proportions of the various isoforms might suggest an increased sensitivity to oxidative stress, metal accumulation and/or alterations in metal homeostasis. Studies in patients with Alzheimer's disease and/or Amyotrophic Lateral Sclerosis are ongoing to identify the status of the individual MT isoforms. This work was supported by VA Research Service and NEIHS 5T32EY10726 to K.K.

METHYL-MERCURY POISONING LEADS TO ENHANCED UPTAKE OF 35S-L-CYSTEINE AND 3H-L-GLUCOSE IN CS7BL/6 MOUSE BRAIN. David Park, Simon Yue and Ben H. Choi. Neuropathology, University of California, Irvine, College of Medicine, Irvine, CA 92717

Previous studies in our laboratory demonstrated that methylmercury (MeHg) poisoning in mice leads to a significant reduction in glutathione (GSH) contents in both brain and liver. We have also shown that an uptake of 35S-L-cystine was significantly enhanced in the brains of CS7BL/6 mice following MeHg intoxication. To examine whether or not a reduction in GSH may underlie enhanced 35S-L-cystine uptake in the brain of MeHg-intoxicated animals, a group of CS7BL/6 mice were injected with 5 mg/kg of methylmercury chloride (MMC) and another group with 1.0 mg/kg of buthionine sulfoximine (BSO), a cysteine-synthesizing inhibitor, for 4 consecutive days. The control animals received physiological saline in place of the drug solution. After the last injection, 35S-L-cystine (1.0 μCi/g) and 3H-L-glucose (1.0 μCi/g) were injected intraperitoneally. Forty five minutes thereafter, the brain and liver were obtained following sacrifice and counted. Both BSO and MMC showed marked GSH reduction in the brain and liver. However, brain uptake of both 35S-L-cystine and 3H-L-glucose was significantly enhanced only in MMC group and not in BSO group as compared to those in control. These results suggest that the enhanced uptake of 35S-L-cystine and 3H-L-glucose in the brain of MMC-poisoned animals may be primarily related to a dysfunction of the blood-brain barrier brought about by MeHg intoxication. (Supported in part by NIH grant ES 02269)
673.9
COMPARISON OF THE TOXICITY OF TRIMETHYL Tin FOR FOUR INBRED STRAINS OF MICE. J. E. Ekuta, M. Tharp and J.C. Matthews. Dept. of Pharmacology, University of Mississippi, University, MS 38677.

The central nervous system toxicity of trimethyl tin (TMT) is characterized by selective nerve and glial cell destruction, particularly granule cells in the hippocampal formation, associated with performance deficits in behavioral tasks used to assess learning and memory. In addition to these rather selective effects, TMT also causes systemic toxicity manifested by a diarrhea, loss of appetite and weight, and death in severe cases. Male mice at 4 months of age from four inbred strains (C57BL/6J, AKR/J, BALB/cByJ, and DBA/2J) were compared for their relative sensitivities to the toxicity of TMT following a single intraperitoneal injection. Body weight and mortality were used as indices for assessing systemic toxicity. The average time taken for the mice from each strain to find the submerged platform (swim time) in the Morris water maze was used to assess spatial learning abilities. Mice of all strains, except BALB/cByJ, died within 36 hours of 3.0 mg/kg TMT administration. At lower doses, the order of sensitivities of the strains based on severity of weight loss was C57BL/6J > AKR/J > DBA/2J > BALB/cByJ. The swim time ranking for the mouse strains was BALB/cByJ > DBA/2J > AKR/J > C57BL/6J. These results demonstrate that different inbred strains of mice exhibit genetically determined differences in their levels of sensitivity to the toxicities of TMT. This finding is an important first step in our quest to understand the role of neurotoxicity in neurodegenerative disease.

673.10
TRIMETHYLNITROSURIE STIMULATED PROTEIN KINASE C TRANSLLOCATION IN DIFFERENTIATED PC12 CELLS. M.D. Kane, G. Palavicino, and G.B. Isaac. Dept. of Pharmacol. & Toxicol., Purdue Univ., West Lafayette, IN.

Trimethyltin (TMT) is a potent neurotoxic agent which causes learning and memory perturbations and culminates in necrotic lesions of the limbic system in rodents and man. Differentiated rat pheochromocytoma (PC12) cells were used to determine the effect of TMT on neuronal signal transduction systems. Treatment of cells with TMT did not increase cytosolic free Ca2+. However, confocal imaging studies of differentiated PC12 cells showed that 10 pm TMT stimulated mobilization of protein kinase C (PKC) from cellular membranes. To characterize this response in more detail, PC12 cells were treated with TMT (0, 5, and 20 pm) or phorbol myristate acetate (PMA; 100 nM) and the extent of PKC translocation to the membrane was determined at 0.5, 4.0, and 24 hrs of treatment. PKC translocation was determined in soluble and membrane cell fractions by western blot analysis using anti-PKC antibodies. The control profile showed greater than 95% PKC in the soluble fraction. Both 5 and 20 pm TMT treatments stimulated a partial translocation of PKC (40%-60%) to the membrane fraction at all time points. PMA treatment stimulated greater than 95% PKC translocation at 0.5 and 4 hrs as well as a complete loss of PKC staining in both fractions at 24 hrs indicating PKC down regulation. TMT stimulated translocation did not result in PKC down regulation at 24 hrs. In separate experiments 100 pm TMT inhibited Ca2+-phosphatidylserine stimulated PKC activity to 27% of control activity as determined by the 32P-acceptor method. These results show that TMT stimulates a partial and sustained translocation of PKC in PC12 cells and inhibits kinase activity. This event appears to be independent of a rise in cytosolic free Ca2+.

673.11
COMPARISON OF INTRACRANIAL AND SYSTEMIC ADMINISTRATION OF TRIMETHYLNITROSURIE ON HIPPOCAMAL DAMAGE. V. Lugo, E. Rasmussen and E. Castaneda*. Behavioral Neurosciences Laboratory, Department of Psychology, Arizona State University, Tempe, AZ 85287-1104.

It has been suggested that accidental exposure to the organometallic toxic trimethyl tin (TMT) produces behavioral deficits that are a result of hippocampal damage, and 2) hyperactivity observed in rats treated systemically with TMT may be useful as a model for attention deficit hyperactivity disorder. In order to further evaluate these postulates, the effects of various doses of TMT infused into the hippocampus were examined. Adult Long Evans rats received bilateral infusions (2 hemispheres) of either vehicle (phosphate buffered saline) or TMT (total of 50, 100, 200, 500 or 1000 mg) into the hippocampus. Two more groups of rats received 8 mg/kg TMT or vehicle systemically (i.p.). At least two weeks later, animals were decapitated and the left hemispheres underwent histological processing for Nissl staining. The hippocampi were dissected from the right hemispheres and prepared for assay of dopamine and serotonin using HPLC-EC. It was found that there was a dose-dependent ablation produced by intrahippocampal application of TMT that was not evident in rats treated systemically. In addition, the two highest intrahippocampal doses produced serotonergic depletions relative to systemically treated rats. These results have important implications for understanding the central mechanism of action by which TMT produces behavioral deficits in human and nonhuman animals. Currently, behavioral measures are being examined for changes induced by intrahippocampal infusions of TMT.

673.12

Glycine (Gly) is an agonist at two neurotransmitter receptor sites, one sensitive to inhibition by Strychne and linked to an Inhibitory Gly-gated chloride channel, and the other a co-agonist site on the N-Methyl-D-aspartate (NMDA) channel complex. In previous reports, we have found a reduction of NMDA receptor density in some cerebral areas of manganese intoxicated mice. In the present study we present evidence that Manganese exposure blocks Gly binding to its receptors by autoradiographic methods. Male albino mice were injected i.p. with manganese chloride (5 mg Mn/kg body weight/day) 5 days per week, during 8 weeks. Control animals received saline injections given plane drinking water. Sacrifices were made on days 14, 25, 26, 27, 28, and 42 (5 animals/day) for the exposed group and days 14 and 28 for the control.

Body weight and water consumption were similar for the exposed and control groups. Gly receptor mRNA levels in dorsal and ventral areas were significantly elevated compared to placebo on days 25, 26, 27, 28 and 42. The Gly receptor mRNA levels were significantly elevated on days 7, 26, 27 and 28 compared to the control. Since there was no difference from control in either Gly receptor mRNA or Gly receptor on day 14, we conclude that the increase in Gly concentrations in this brain region is not due to an increase in Gly receptor expression. These data suggest that manganese increases Gly receptor mRNA levels in the rat brain and may play a role in the development of manganese induced neurotoxicity.
AN IN VITRO MODEL TO STUDY THE RELATIONSHIP BETWEEN IRON AND PROTEIN OXIDATIVE MODIFICATION. S.H. Hackett*, R.B. Sturz*, E. Ferreira*, W. Van Gelder†, J.B. Cazana†. 1st Dept. of Neurosciences & Anatomy, 2nd Dept. of Pathology, LSUHSC, New Orleans, LA. 70112.

The relationship between iron toxicity and oxidative damage to proteins is of increasing interest to neurologists and neuroscientists. We present a model to study this relationship in an in vitro system. Experimental conditions were designed to induce oxidative stress and determine the specificity of iron-mediated protein modifications. Iron was supplied to differentiated PC12 (neuronal-like) cells and to primary cultures of adult rat cortical neurons. The deprenyl-protected, rat nigrostriatal explant cultures were also used. The iron source (EDTA-Fe III) was added to the culture medium at different concentrations. The results indicated that iron and iron chelators (DMSA, BAPTA, MnCl2) can modify proteins (e.g. BS integrin, L-arginine synthase, FGF receptor). The iron and chelator dosage dependence of the protein modifications was assessed. The modifications occurred with a high degree of specificity, with limited modification of non-iron-containing proteins. This model could be used to assess the role of iron in the pathogenesis of neurodegenerative disorders such as Parkinson's disease and to assess the role of iron chelation therapy for the treatment of these diseases.


A process of metabolic activation is thought to be the first step leading to the toxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydroxypyridine (MPTP). This conversion forms the toxic 1-methyl-4-phenylpyridinium (MPP+) metabolite and in the brain is most likely mediated via glial monoamine oxidase (MAO) type B (MAO-B) inhibition. However, does not completely block MPTP conversion to MPP+ since MPP+ is still formed in the presence of MAO inhibitors both in vitro and in vivo. The purpose of the present study was to determine whether that iron may act as a catalyst for the MAO-independent bioactivation of MPTP. Primary cultures of mouse astrocytes were treated with both the MAO A inhibitor clorgyline and the MAO B inhibitor deprenyl prior to the addition of MPTP. Production of MPP+ was reduced (to approximately 15%), but not completely by MAO inhibition. This MPP+ production appeared to be iron-dependent since it was decreased (30 to 50%) by iron chelators, deferoxamine or phenanthroline, and was enhanced (by more than 50%) in the presence of ADP. Data indicate that oxidation via MAO is the primary but not the only pathway of MPTP bioactivation and that transition metals may contribute to the generation of the toxic MPP+ metabolite in biological systems.

NETROTOXICITY: METALS

THURSDAY PM

673.15

673.17

673.19

673.20
673.21 LEAD REDUCES CALCIUM ENTRY WITHOUT PASSING THE CELL MEMBRANE OF MAMMALIAN NEURONS: FURA 2 MEASUREMENTS. D. Busselberg*, R. Donnem, I. Wunder, and H. L. H. H. Physiology II and BMFZ, Heinrich-Heine-University, P0B 100177, D-40001 Düsseldorf, Germany.

Lead is an ubiquitous but neurotoxic element. Recent work has demonstrated that its neurotoxicity is partly due to actions on voltage activated calcium channel currents (Busselberg et al., 1994, J. Neurophysi ol., 71). It reduces calcium channel currents with an IC₅₀ of ~0.5 µM. Here we prove our prediction that the rise of the intracellular calcium concentration is prevented by lead. Dorsal root ganglion neurons of rats were depolarized by exposure to 50 mM potassium and the rise of internal calcium was measured with Fura 2. Lead reduced this rise in a concentration dependent manner, with a threshold concentration of 0.25 µM and ~5 µM lead reduced the calcium entry by 280%. The effect was most likely due to voltage activated calcium channels but does not pass through the channel itself.

673.22 EFFECT OF LEAD INTOXICATION ON SYNUVIA DEHYDROGENASE ACTIVITY AND THIAMINE CONTENT IN THE BRAIN OF RAT. K. I. and Y., H. and I. E. College of Pharmacy, Seoul National University, Seoul 151, Korea

Thiamine has been reported to reduce the toxic manifestations of lead at lead level in tissues. Concomitantly, lead intoxication may affect thiamine status and thiamine-related biochemical responses. In this study, it was tested if lead intoxication could change thiamine content and the activity of pyruvate dehydrogenase in the brain and administration of thiamine could prevent the toxic manifestations of lead intoxication.

Five groups of Wistar rats were prepared: 1) control group, 2) lead group, 3) lead plus thiamine treated group, 4) pyruvithiamine treated group, and 5) pyruvithiamine treated group. Each group of animals was divided into five subgroups based on age, 3, 7 and 16 weeks of age. Lead was administered by drinking drinking water containing 0.2% lead acetate and thiamine was administered via drinking water. It was divided in 3 groups according to the period of treatment: 3 days, 7 days, and 16 days. All animals were treated with either lead acetate or water and thiamine for 16 days. As a result of this treatment, it was observed that lead intoxication lowered the activity of pyruvate dehydrogenase in all brain regions and the activity increased significantly in the thiamine treated group. Thiamine intake in brain regions of lead treated group was significantly lower than those of control group, and those of lead plus thiamine treated group was significantly decreased from those of lead treated group. Thiamine content in brain regions of lead treated group was significantly lower than those of control group, and those of lead plus thiamine treated group was significantly higher than those in lead treated group.

The results from the present study suggest that lead neurotoxicity including convulsion following lead intoxication in rats may be mediated at least in part through the changes of thiamine status and thiamine related factors.

673.23 CHRONIC LOW-LEVEL LEAD STIMULATION OF NEURAL CELL SYNALIATION STATE: Fleur D. Hayes & Kieran C. Broer*. Dept. of Pharmacology and Clinical Pharmacology, University of Dundee, Ninewells Medical School, Broughton Road, Dundee, DD1 9SY, Scotland, U.K.

Lead is a neurotoxic agent which acts preferentially on the developing nervous system. Although the molecular mechanisms underlying the actions of lead are not well understood, it has been suggested that it may act to disrupt the processes of neurite elaboration and consequent synapse formation. The neural cell synaliation state is precisely regulated at this stage of development and may thus provide a possible target for lead toxicity.

Hippocampally-derived neuronal cell hybrids were cultured in the presence of low-level lead (10⁻⁴ to 10⁻¹M) for 72 hours, the cells harvested and the activity of the sialyltransferase enzyme determined. Sialyltransferase activity was stimulated three-fold by very low lead levels in cells derived from both embryonic day 15 and postnatal day 1, the former giving more reproducible lower lead levels. Lectin blot analysis demonstrated an increase in the sialylation state of specific glycoproteins. Although the nerve cell adhesion molecule, NCAM, is a developmentally-regulated sialylcoeprotein, there was no evidence for a lead-induced re-expression of the polysialylated form of the protein. This results demonstrate that neural cell glycosylation is susceptible to chronic low-level lead exposure, and this may contribute to the upshift in synapse formation associated with lead-induced neurotoxicity.

This study was supported by a Wellcome Toxicology Studentship to F.D. H.

673.25 LEAD INDUCES A BIPHASIC GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) RESPONSE IN RAT BRAIN. Z.L. Gona, A.R. Little, Han, E. Fawal, H.I. Evans*, Institute of Environmental Medicine, New York University Medical Center, Tuxedo, NY 10987

GFAP was used to test whether repeated exposure to lead (Pb) produces a different pattern of change in astrocytes at early stages of exposure, especially at postnatal stages, and at higher doses measured Pb were different than at higher doses which produce overt toxicity. Male F344 rats (~42 days old) received either lead acetate (Pb: 50 or 1000 ppm) or trimethyl lead (TML: 8 or 16 ppm Pb) in their drinking water for 7 or 14 days. Although TML produced much more toxic, as shown by decrease in body weight, both Pb and TML produced a similar biphasic pattern of increase in brain GFAP. As a result of this treatment, Pb occurred at lower levels of exposure (50 ppm Pb) or at early stages of exposure (day 7 of 8 ppm TML); increases in GFAP occurred at higher levels of exposure (1000 ppm Pb or 15 ppm TML) or at later stages of exposure (day 14 of 8 ppm TML). Dose-response was observed in hippocampal rats of rats exposed to TML and in cerebral cortex and cerebellar rat exposed to Pb. The observed biphasic change in GFAP levels during Pb exposure suggests that astrocytes play a role in the brain's earliest response to neurotoxic metals. We propose a two-stage astrocytic gliosis occurs in response to neuronal damage as indicated by increased GFAP. Supported by the American Institute for Cancer Research and Grants ES-04895.


The purpose of this study was to determine if postnatally lead-treated kittens have a greater susceptibility to bicuculline-induced seizure initiation. Kittens were treated with 20 mg/kg lead acetate solution daily by oesophageal intubation for seven days postnatal. Control kittens were maintained as the same except they were administered 20 mg/kg sodium acetate solution. The kittens were allowed to age to one year during which time they were observed for any behavioral abnormalities. No spontaneous seizures were seen in either group. The kittens were anaesthetized with an IM injection of a solution containing 50 mg Ketamine, 5 mg Xylazine and 1 mg Acepromazine and the femoral vein was cannulated for delivery of drugs. Small holes were drilled into the skull and electrodes were placed on the frontal and parietal cortex. A tracheotomy was performed, auffed pediatric ET tube inserted and the kitten respirated with 55% O₂/45% CO₂. The animals were paralyzed with 150 mg/kg Pancuronium. The EEG was recorded during this procedure to determine baseline neural activity. Bicuculline (0.15 mg/ml) was infused via the femoral canula with a Harvard infusion pump at a rate of 0.20 ml/min. Seizure initiation as measured by EEG was EEG during a 4 min interval. The EEG was recorded during this procedure to determine baseline neural activity. Bicuculline (0.15 mg/ml) was infused via the femoral canula with a Harvard infusion pump at a rate of 0.20 ml/min. Seizure initiation as measured by EEG was later observed. The bicuculline was administered every 10 minutes and the response was noted. It was observed that the bicuculline in experimental and 0.36 mg/kg bicuculline in control subjects. The brain of these kittens will be processed by the Golgi-Cox method to determine if cerebral cortical neurons are hyperpyrexic. Hyperpyrexia in lead-exposed kittens have been described previously by our laboratory. It will be of great interest if seizure susceptibility is correlated with the hyperpyrexia condition. Predictions towards seizure activity may be implicated in neurobehavioral aberrations seen in lead-exposed kittens.
674.5 LONG-TERM SURVIVAL OF MPTP-TREATED AGING MICE AND THE ROLE OF GIANT, X. L. CHEN, AND M. G. GUYA. Department of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY 40202.

Previous studies from this laboratory have shown that MPTP treatment in the young adult mice significantly reduces the number of dopaminergic neurons in substantia nigra (SN). Six months after MPTP treatment, there is a significant recovery of the dopaminergic neurons. The present studies were undertaken to examine plasticity of aging dopaminergic neurons in the SN following toxic insult. Aging male C57BL/6 mice (18 month old) received MPTP in an dose of 25 mg/kg (i.p.) and were examined 3 days, 2 weeks, and 6 months after MPTP treatment. Control and MPTP-treated mice were anesthetized and perfused. Intact brains were cryoprotected with 4% paraformaldehyde in 1.5% sucrose in 0.1 M phosphate buffer. Frozen 40 um thick sections were cut through the entire brain. Adjacent sections were stained immunocytochemically with tyrosine hydroxylase (TH) antibody and 6-OHDA fluorescent (MPP)-immuno reaction. The number of TH-positive neurons was quantitated in the SN. The results show a significant reduction of TH-positive neurons in SN at 3 days after MPTP compared to the control group. The number of TH-immunoreactive cells was reduced to 45% of the control level at 6 months after MPTP treatment. The recovery even up to 6 months after MPTP treatment compared to the young adult mice. Since glia convert MPTP into its toxic metabolite MPP+ which is increased glial fibrillary acidic protein (GFAP) immunoreactivity is increased in the old aging mice. It is possible that glia play a role in the reduced ability of aging mice to regenerate following toxic insult. Supported by USPHS grant NS 24921 to MG.

674.6 INDUCTION OF C-FOS FOLLOWING MPTP TREATMENT IN THE YOUNG ADULT AND AGING MICE. M. GUYA*, C. HITCHEN, AND X. L. CHEN. Department of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY 40202.

Previous studies from this laboratory have shown a loss of dopaminergic neurons in the substantia nigra (SN) and decreased levels of dopamine in the striatum following MPTP treatment in the young adult mice. By six months after MPTP, there is a significant recovery in the SN of young adult mice compared to the aging mice, treated in a similar manner. Immediate early genes that are induced by molecular signals and after transcription of other genes, are activated in the rat striatum of cocaine and amphetamine treatment. The present studies examined the effects of MPTP treatment on the induction of c-fos in the aging mice which was examined by c-fos immunostaining. Frozen sections of the aging mice were cryoprotected with 4% paraformaldehyde in 1.5% sucrose in 0.1 M phosphate buffer. Frozen 40um thick sections were cut from the entire brain. Adjacent sections were stained immunocytochemically by tyrosine hydroxylase (TH) antibody and c-fos fluorescent (MPP)-immuno reaction. The number of TH-positive neurons was quantitated in the SN of aging mice. The results show a significant increase of c-fos in the young adult mice 30 minutes after MPTP treatment. The induction of c-fos was not observed in the aging mice. The results are consistent with the hypothesis that oxidant formation may be induced by MPTP through the release of DA, the observation that c-fos is produced in an area displaying no significant DA release suggests that a second mechanism is also involved. The protective potential of MPTP for the aging mouse, which is not observed in the young adult mice, may be due to an increased recovery of protein synthesis in the aging mice treated with MPTP. Supported in part by USPHS grant NS 24921 to MG.
**NEUROTOXICITY: STRIATUM**

**674.7**


A single systemic injection (0.3 mg/kg; i.v.) of MPTP, a neurotoxin that induces a Parkinsonian-like syndrome, produces a progressive high amplitude swelling of tyrosine hydroxylase (TH) positive neural processes in the proximal third of the nigrostriatal (NS) pathway of dog. This swelling is evident one day after an injection; and by 14 days, many profiles reach a diameter of 30µ or more. In parasagittal sections, the swellings have a club-like appearance with the head pointing rostrally towards the striatum and the handle tapering towards the nigra. The club-like swellings are acidophilic and are immunopositive for the non-phosphorylated neurofilaments. The periphery of the swellings reacts positively for antibodies against myelin basic protein. Examination of the TH-positive swollen profiles with the electron microscope reveal that they are myelinated axons. Thus, unlike rats whose NS-neurons are unmyelinated and survive exposure to MPTP, NS-neurons of the dog are myelinated and are killed by MPTP. We hypothesize that the degree of myelination is positively correlated with a DAergic neuron's ability to recover from a MPTP insult.

**674.8**


The present study sought to map and quantify the number and location of midbrain dopaminergic (DA) neurons that are susceptible to degeneration following MPTP treatment, and determine whether the vulnerable cells contain the calcium-binding proteins calbindin-D28k (CALB) or calreitin (CALR). Brain sections from adult male FVB mice were processed for simultaneous two-color fluorescence immunocytochemistry for tyrosine hydroxylase (TH) and CALB, and TH and CALR. Three groups of 3 FVB mice received 4X25 mg/kg MPTP (at 2h intervals) either on Day 1 alone, Days 1 and 3, or Days 1,3,4,5 for cumulative doses of 100, 200, and 300 mg/kg, respectively. Mice were sacrificed 7 days after the last injection. There was over an 80% reduction in striatal dopamine concentrations in all three treatment groups, however, DA cell loss was only observed after the 300 mg/kg dose. There was an average of ~40% cell loss that was confined to a mid-rostral caudal portion of the substantia nigra pars compacts, in a region largely devoid of CALB or CALR in the DA neurons.

**674.9**

**A NONINVASIVE APPROACH TO TEST DOPAMINE OXIDATION BLOCKADE BY MPTP AND MPP+ NEUROTOXINS IN RATS. S.P. Bagchi*, Nathan Klene Inst.for Psychiatric Res., Orangeburg, NY 10962**

MPTP and MPP+ may induce Parkinson's disease syndrome and also impair oxido-reduction of dopamine (DA) by mouse striatum. A noninvasive approach based upon urinary DA and DOPAC levels to test oxidation defect induced by MPTP, MPP+ or other causes may be clinically useful. Neurotoxin was administered i.p. to rats put in metabolic cages for collecting urine over 1 N HCl for subsequent 0-12, 12-24 or 0-24 hr. time periods. Urinary DA and DOPAC were quantitated by published methods employing gas chromatography. A single 5.0 mg/kg dose of MPTP or MPP+ acutely depressed urinary DOPAC level and DOPAC/DAA ratio compared to saline control. Reserpine (RES) (2.5 mg/kg) acutely raised DOPAC level and DOPAC/DA ratio and these effects were reversed by either MPTP, MPP+, or paraglyine (30.0 mg/kg), but not by the MPP+ congener paraquat (5.0 mg/kg), given 1 hr. before RES. At 13 days after a single MPP+ dosage, DOPAC level and DOPAC/DA ratio did not differ from the control. RES effects, however, were still blunted at 13 days. Five d 5.0 mg/kg MPP+ dosages had powerful effects at 0-24 hrs. following the last dosage but the values reverted to normal by 6 or 13 days; at 27 days, reserpine revealed impaired oxidation of DA in conclusion. MPP+ may cause an acute as well as a long-lasting impairment of peripheral DA oxidation and the latter may become apparent only in reserpenized animals. Also, the RES induced sensitivity is due to an enlargement of the limiting free DA level at MAO site rather than because of the release of stored neurotoxin. Supported by Office of Mental Hygiene, State of New York.

**674.10**

**EVALUATION OF THE EFFECTS OF HEAT STRESS ON THE NEUROTOXICITY OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDRODIPYRIDINE (MPTP) IN C57/BLN MICE. T.K. Freydenberg*, W. Siskor, Jr. and S.F. As. Division of Neurotoxicology, NCTR/FAA, Jefferson, AR 72079 and Dept of Biochem. & Mol. Biol., UAMS, Little Rock, AR 72205**

MPTP is known to cause neurotoxicity in rodents and nonhuman primates. The present study was designed to determine whether heat stress, with the subsequent induction of stress proteins, 24 hr prior to dosing with MPTP might have an effect on striatal dopamine depletion. Adult male C57/BLN mice were dosed with 4 x 10 mg/kg, ip MPTP at two hr intervals and sacrificed without previous heat stress. Animals were sacrificed at 24 hr after the last dose. Stratal dopamine and its metabolites were measured by HPLC/EC and stress proteins by SDS/PAGE. MPTP produced 70% depletion of dopamine in the striatum of non-heated stressed animals, whereas in heat stressed animals striatal dopamine depletion was 83%. Animals subjected to heat stress alone or injected with saline showed no depletion of striatal dopamine. Stress protein induction could not be detected by SDS/PAGE or immunoblotting in either the striatum or the substantia nigra. These results suggest that heat stress has lasting effects which enhance the neurotoxicity of MPTP.

**674.11**


The effects of condensation products of dopamine and indoleamines on the activity of tryptophan hydroxylase (TPH) were evaluated to determine the structures associated with modulation of this enzyme activity. The compounds having a catechol structure, such as 6,7-dihydroxy-1,2,3,4-tetrahydroxyxosin-TGD, were shown to potentially inhibit the activity of the enzyme prepared from the rat brain. The inhibition was non-competitive in terms of both the bipterin cofactor and the substrate L-tryptophan. The substitution on 1 or 2 positions of 6,7-dihydroxy-TGD did not affect their inhibitory capacities to TPH activities. Among these compounds, a charged substance, 1-methyl-2(2-methyl-6,7-dihydroxyxosin-TGD) was shown to be the potent inhibitor; the Ki values were 0.88 ± 0.17 and 0.64 ± 0.08 µM (mean ± SD) in terms of the substrate and cofactor, respectively. On the contrary, the condensation products of indoleamines scarcely affected TPH activities. 1-Methyl-TGD, MPTP, and MPP+ were almost inactive. These results indicated that the catechol structure recognized and combined with TPH at the binding site different from that of the charged substrate and the dopamine-derived substance enhanced the affinities to TPH. The inhibitory capacities of these compounds would be enhanced by the cyclizing reaction of dopamine. (Supported by a Grant-in-Aid for Scientific Research on a Priority Area #1303 of Japan).

**674.12**


Recent studies show that p-carbolinium ions (BC+) resemble the synthetic parkinsonian toxicant, MPP+, with respect to structure and neurotoxicity (Brain Res. 570, 154, 1992) and are identified in human brain by the GC/MS analysis (Brain Res. 610, 90, 1993). These potential bioactivated neurotoxicants, 2-N-methyl-p-carbolinium (2-MeBC+) and 2,8-N-dimethyl-p-carbolinium ions (2,8-Me2BC+) were analyzed in the lumbar cerebrospinal fluid (CSF) of 16 parkinsonian and 16 non-parkinsonian patients using HPLC/fluorescence detection. The levels of BC+ ions in the lumbar CSF of parkinsonian patients were significantly higher than those in non-parkinsonian individuals. On the other hand, there was no significant difference in the CSF levels of simple p-carboliniums, which are precursors of BC+, between parkinsonians and non-parkinsonians. These results strongly support the hypothesis that "bioactivated" carbolinium ions, which are 2-MeBC+ and 2,8-Me2BC+, could be endogenous causative factors in Parkinson's disease. (Supported by a Grant-in-Aid for Scientific Research on a Priority Area #1303 of Japan).
NEUROTOXICITY: STRIATUM

674.13

We are comparing the effects of a single unilateral injection of the neurotoxin 6-hydroxydopamine (6-OHDA) into the striatum versus the substantia nigra on ipsilateral rotational responses to D-amphetamine injection. Seventeen out of nineteen rats injected ipsilaterally to D-amphetamine (3.0 mg/kg) when tested at 13-18 days following injection of 25 μg (1.5 μl) of 6-OHDA into the right striatum. Repetition of the 1 hr. rotational test at between 25 and 53 days post lesion revealed a decreased response in twelve of the fourteen original positive responders. Ipsilateral rotations were significantly (P < .005) reduced to a mean value (+ SEM, 189 ± 50) which was only 43% of the original response (474 ± 49). Substantia nigral-lesioned rats (N=6) that tested positive at 10-21 days post lesion showed a mean response upon retesting at 61-79 days post lesion (606 ± 63) which was 160% of their original value (472 ± 110). These findings demonstrate a limited behavioral recovery in striatal but not substantia nigral 6-OHDA-lesioned rats. Supported by a BRSG grant to M.L.L.

674.15

There has been considerable interest in the possibility that perturbations of energy metabolism play a role in the nigrostriatal degeneration. Methamphetamine (METH) has been shown to cause a long-lasting dopaminergic neurotoxicity in the striatum. Although it has been suggested that endogenous dopamine (DA), excitatory amino acids, and oxidative stress are involved in METH toxicity, the relationship between energy impairment and the toxic effects of METH on dopaminergic neurons remains to be explored. In the present study, experiments were conducted to investigate the effects of METH on mouse brain ATP levels and the relationship between METH-induced changes in striatal dopamine and ATP concentrations. C57BL/6 mice were treated with either a single or 4 doses of METH (10 mg/kg, i.p., given at 2 hr intervals) and killed by microwave irradiation of the brain at either 1.5 hr or 1 week after the last injection. ATP levels were measured in the striatum, hippocampus and cerebellar cortex, and striatal DA levels were also determined. Neither striatal ATP nor DA concentrations changed after a single injection of METH, but both were decreased after 4 doses of METH. No changes in ATP were observed in the hippocampus and cerebellar cortex. In a second set of experiments, an intraperitoneal injection of 2-deoxyglucose (2-DG, 1 g/kg), an inhibitor of glucose uptake and utilization, was given 30 min prior to the last 2 injections of the 4 doses of METH. 2-DG significantly potentiated METH-induced striatal ATP loss at 1.5 hr and DA depletions at 1.5 hr and 7 days. These results indicate that a toxic regimen of METH results in a selective energy impairment in striatum, and raise the possibility that energy impairment plays an important role in METH-induced dopaminergic neurotoxicity.

675.1
Excitotoxic mechanisms in cultured neuronal and glial cells overexpressing Cu/Zn-superoxide dismutase (Cu/ZnSOD). O. Bar-Peled* and Y. Groner, Molecular Genetic and Virology Dept., The Weizmann Institute of Science, Rehovot, Israel 76100.

To study the involvement of Cu/ZnSOD overexpression in excitotoxic cell death, susceptibility of cortical areas and cord neurons of transgenic-Cu/ZnSOD (TgHS) mice to Kainic acid (KA) was investigated. Glial-free neuronal cultures grown in medium without serum, were exposed to KA for 15-20h and surviving cells monitored by counting the number of Neurons Specific Enolase positive cells. As previously recorded in mixed neuronal and glial cultures, 50 and 100μM KA led to 1.5-2 fold increase in cell death when glial-free cultures from TgHS were compared to control mice. The role of glial cells in this differential vulnerability was studied. Astroglial cultures were prepared and uptake of [3H]-glutamate was measured. 50% increase in glutamate uptake per cell was found in control cultures as compared to cultures from TgHS mice. Glutamate uptake is an energy dependent process driven through a sodium gradient maintained by Na+/K + ATPase. To examine whether lower activity of the Na+ pump in TgHS astrocytes was involved in the reduction of glutamate uptake, ouabain, an inhibitor of Na+ pump was added. At 10μM it caused a reduction of glutamate uptake in both TgHS and control astrocytes, however, the inhibition in TgHS cells was 40% larger. When subjected to KA, TgHS spinal-cord neurons were more susceptible than control neurons. Upon incubation with Osmolite (20mM) for 20h, 60% more spinal neurons died in the TgHS cultures. Electron microscopy analysis of KA-treated neurons revealed condensed chromatin and abundant vacuolization in the cytoplasm, indicating that KA-induced cell death is mediated by apoptosis.

675.2

Oxygen deficiency and chronic dexamethasone treatment are known to cause damage to the central nervous system. Cortical regions, such as the hippocampus, are vulnerable to these insults. Several groups were formed based on treatment and timing. Rats received oxygen deficiency treatment on the day of birth (P0), at P7, or at both P0 and P7. Dexamethasone treatment was received on the day and without oxygen deficiency treatment(s). There were 7 treatment groups including the controls. Oxygen deficiency consisted of 3 treatments each separated by 30 minutes for both the P0 and P7 apps. At P0, oxygen deficiency criterion was reached when the rat demonstrated either 2-15sec or 1-45sec intergasp intervals while in the chamber. At P7, the criteria was 2-15sec or 1-30sec intergasp interval. Dexamethasone treatment was given for 7 consecutive days at 5mg/kg body weight. Openfield activity measures and performance on the holeboard memory task were assessed. Preliminary results indicate that P7 oxygen deprivation caused the greatest behavioral disruption. Rats of groups that received a P7 oxygen deficiency treatment showed a decrease in activity, and were less efficient in completing the memory task. Histological analysis are being conducted. (Conducted under NIH Guide for care and Use of Laboratory Animals).

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Exposure of the mammalian brain to ionizing radiation, whether as part of a cancer therapy or due to industrial nuclear accidents, causes severe cerebral damage. This study was performed to evaluate the effect of the antiglutamate neuroprotective, riluzole, on the acute structural and behavioural damage inflicted by a dose of 2.5 Gray to the brains of 15 day old rats. Rats were irradiated from a Cobalt 60 source. They received riluzole at a dose of 8 mg/kg i.p., 20 minutes after the start of the irradiation. Six hours later animals were perfused and brains removed. The hippocampus was cut in 1 μm sections and stained with toluidine blue. The proportion of pyknotic cells per 1000 cells in the granular and subgranular layers of the dentate gyrus was counted in three non-similar slides. In other animals behaviour was examined in an open field.

Irradiation at 2.5 Gray caused pyknosis in neurons in the dentate gyrus. Riluzole reduced the number of pyknotic cells in irradiated rats, with a dose-dependent and statistically significant protection seen between 1 and 4 mg/kg i.p. At 10 mg/kg 18.0±0.7% of neurons pyknotic compared to vehicle-treated controls with 24.6±0.8% cell death; n=10/group, p<0.05. In the open field irradiated animals showed signs of lethargy, unco-ordinated movements and the number of squares crossed in the field fell. Riluzole significantly increased mobility to levels of non-irradiated siblings from doses of 2 mg/kg i.p (squares crossed per 3 minutes: 4.6±0.8, 13.3±2 and 19.7±3.1 for irradiated, irradiated plus riluzole at 2 mg/kg respectively). Riluzole may have a neuroprotective agent for use after accidental or deliberate exposure to ionizing irradiation.

675.5 NEUROTOXICITY ASSOCIATED WITH OXYGEN RADICALS: GENERATION IS EXACERBATED BY GLUCOCORTICOIDS. L.J. McIntosh* and R.M. Saposnik. Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305, and Univ. of British Columbia, Vancouver, B.C., Canada

Glucocorticoids (GCs) are secreted by the adrenal in response to stress, and are also used clinically to control inflammation and autoimmune disorders in millions of people annually. Thus elevated GC levels are not uncommon in populations at risk for neurologically injured tissue and is it known that GCs exacerbate various forms of neuronal injury. GCs act on cellular pathways relevant to oxygen radical function, but it is unclear whether GCs are an independent oxygen radical toxicity. To test this hypothesis we found that glucocorticoids may decrease the ability of neurons to protect themselves against reactive oxygen species, we examined the effect of the glucocorticoid dexamethasone on primary neuronal cultures. Susceptibility to adirymycin was determined by cell counting after MAP-2 staining, and correlated with biochemical measurements. After establishing a dose-response curve for adirymycin, we determined that the lipophilic antioxidants Trolox and butylated hydroxytoluene were effective in decreasing the adirymycin toxicity, indicating that adirymycin was in fact producing toxicity through reactive oxygen p-chains.

Increasing GC concentration in the culture media (up to 10-6 M) exacerbated the toxic effect of the adirymycin dose-ncg on the hippocampal and cortical cultures (brain regions with high α: moderate concentrations of corticosteroid receptors, respectively) worse compared. Our results indicate that GCs may be directly involved in the pathways that mediate oxygen radical formation, and further study of the connections between oxidative stress and glucocorticoids could indicate useful points at which antioxidant intervention will decrease neuronal death after a neurologically insult.


Using microdialysis techniques, we applied salicylate hydroxide as an in vivo trapping procedure to monitor the time course of 2,3- and 2,5-dihydropyridine (DBH) generation in the striatum following whole-body irradiation. Male Sprague-Dawley rats were exposed bilaterally to 10 Gy of Co radiation at a dose rate of 10 Gy/min. Microdialysis was performed in anesthetized rats either immediately or 1, 2, or 3 days after irradiation. The probe was inserted into the right striatum and perfused with artificial cerebrospinal fluid (ACSF) at a flow rate of 1 ml/hr. Sample collection every 15 minutes was performed in 1 hr after probe insertion. After the initial 2 samples were collected, in vivo trapping was initiated by perfusing 1 ml Na salicylate in ACSF through the microdialysis probe. Formation of 2,3- and 2,5-DHBA was detected by HPLC-EC. The mean concentrations of DHBA in the initial 2 samples were taken as baseline, and their concentrations after salicylate perfusion were expressed as percentage of baseline. The respective baselines for 2,3- and 2,5-DHBA in sham controls were 123.4 ± 17.6 and 290.5 ± 31.7 fmol/μl (n=16). There was no significant difference between the baselines in irradiated rats and sham-irradiated controls. The concentrations of DHBA increased significantly (p<0.05) following salicylate perfusion, and reached plateaus by 45 min. A significant difference is the striatal 2,5-DHBA levels was observed between irradiated rats and sham controls by 3 day after irradiation. This study demonstrates that there are increased levels of OH radicals found in the rat striatum 3 days after exposure to ionizing radiation.


During ischemia, peroxynitrite may be a toxic intermediate which forms in vivo in nitric oxide condenses with superoxide. Alone, peroxynitrite appears to directly react with and reduce myoglobin and porphyrins. At physiological pH, peroxynitrite rapidly (within seconds) decomposes to species with 'OH and NO3- character. These reactive species are shown to initiate lipid peroxidation, haemolysis aromatic residues, and nitrite aromatic residues. This reactivity may contribute to differential toxicity in vivo and in vitro. Tirilazad mesylate (TM) is a lipid-soluble antioxidant shown to inhibit the toxic effects of peroxynitrite. It is an effective therapy in a variety of neurotraumatic models of CNS injury, and is currently undergoing human clinical evaluation in stroke and spinal injury. This study was designed to investigate the cytoprotective properties of TM in a cerebellar granule cell model of toxicity involving treatment with peroxynitrite. Cytoprotective performance of TM was based on viability assessed by a variety of assays: mitochondrial dehydrogenase activity, mitochondrial dehydrogenase, and antioxidant uptake), blockage of lipid hydroperoxide generation, and blockage of nitrotyrosine formation. Results show that viability measurements associated with peroxynitrite toxicity were improved with TM treatment. Interestingly, TM was found to be an effective cytoprotectant (EC50 = 100 μM) even as a post-treatment following exposure of cells to peroxynitrite. Similar post-treatment with supradose concentrations of TM was able to rescue 95% of the cells that had produced any cytoprotection. These and other parameters of peroxynitrite-dependent cytotoxicity will be described with respect to TM cytoprotection.


Using microdialysis techniques, we applied salicylate hydroxide as an in vivo trapping procedure to monitor the time course of 2,3- and 2,5-dihydropyridine (DBH) generation in the striatum following whole-body irradiation. Male Sprague-Dawley rats were exposed bilaterally to 10 Gy of Co radiation at a dose rate of 10 Gy/min. Microdialysis was performed in anesthetized rats either immediately or 1, 2, or 3 days after irradiation. The probe was inserted into the right striatum and perfused with artificial cerebrospinal fluid (ACSF) at a flow rate of 1 ml/hr. Sample collection every 15 minutes was performed in 1 hr after probe insertion. After the initial 2 samples were collected, in vivo trapping was initiated by perfusing 1 ml Na salicylate in ACSF through the microdialysis probe. Formation of 2,3- and 2,5-DHBA was detected by HPLC-EC. The mean concentrations of DHBA in the initial 2 samples were taken as baseline, and their concentrations after salicylate perfusion were expressed as percentage of baseline. The respective baselines for 2,3- and 2,5-DHBA in sham controls were 123.4 ± 17.6 and 290.5 ± 31.7 fmol/μl (n=16). There was no significant difference between the baselines in irradiated rats and sham-irradiated controls. The concentrations of DHBA increased significantly (p<0.05) following salicylate perfusion, and reached plateaus by 45 min. A significant difference is the striatal 2,5-DHBA levels was observed between irradiated rats and sham controls on day 3 after irradiation. This study demonstrates that there are increased levels of OH radicals found in the rat striatum 3 days after exposure to ionizing radiation.
671.9

INHIBITION OF NEURONAL NITRIC OXIDE SYNTHASE (NOS) PROTECTS AGAINST NEUROTOXICITY PRODUCED BY 3-NITROPROPIONIC ACID, MALONATE, AND MPTP. J.B. Shafer*, R.T. Mattison, D.R. Hendrson, M.F. Heal. Neurology Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

Nitr oxide (NO) is synthesized from the guanidino nitrogen of L-arginine and molecular oxygen by nitric oxide synthase (NOS). At least three isoforms of NOS have been identified: constitutive (i) neuronal and (ii) endothelial isoforms, and (iii) an inducible isoform originally isolated from macrophages. Nitrotoxidine (7-NI) was recently found to be a potent and selective inhibitor of rat cerebellar NOS activity after peripheral administration (Institute of Neurobiology, 110: 225-228). We examined whether treatment with 7-NI is protective against neuronal injury produced by mitochondrial toxins, which are known to cause secondary excitotoxic cell death. Lesions produced by stratal injections of malonate, a reversible inhibitor of succinate dehydrogenase, were dose-dependently attenuated by 7-NI treatment in rats. 3-Nitropropionic acid is an irreversible inhibitor of succinate dehydrogenase which causes selective striatal lesions closely resembling Huntington's disease. 7-NI treatment significantly delayed the occurrence of 3-nitropropionic acid lesions in rats. MPTP induced depilation of depaminergic neurons was significantly blocked by 7-NI treatment in mice. Our data indicate that NO plays a role in toxicity of neurotoxins which impair oxidative phosphorylation. Inhibitors of neuronal NOS may be therapeutically useful in neurological diseases hypothesized to be related to an impairment of energy metabolism, such as Parkinson's disease and Huntington's disease.

676.1

EFFECTS OF COMBINED GESTATIONAL AND LACTATIONAL EXPOSURE TO COPLANAR PCBs OR TCDD ON SPATIAL LEARNING AND MEMORY IN RATS. R.W. Sea, J. Mohnthag, B.W. Moore, R.F. Peterson, and S.L. Schantz. Institute for Environmental Studies and Neurotoxicology Program, Univ. of Illinois Urbana, IL 61801 and School of Pharmacy, Univ. of Wisconsin, Madison, WI 53706.

In a previous study, monkeys showed facilitated spatial learning following combined gestational and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Here, we tested spatial learning and memory of rats following gestational and lactational exposure to coplanar polychlorinated biphenyls (PCBs) or TCDD. Time-mated Sprague-Dawley rat dams were gavaged with 2 or 8 mg/kg PCB 77 (3,3',4,4',5-pentachlorobiphenyl), 0.25 or 1.00 ug/kg PCB 126 (3,3',4,4',5-hexachlorobiphenyl), 0.025 or 0.1 ug/kg TCDD, or corn oil vehicle for 10-16 gestation. Litters were culled to eight on day two and weaned on day 21. Beginning on day 80, one male and one female from each litter were tested on an eight-arm radial maze with all arms baited. The animals were tested for 20 sessions, and the data were averaged into blocks of five sessions. All groups had similar numbers of errors (entries into arms already visited) in the first block of sessions, but the TCDD-exposed males subsequently displayed a pronounced dose dependent decrease in errors relative to controls. The decrease in number of errors was statistically significant at both TCDD doses in the second block and at the higher dose in the third and fourth blocks. The pattern of effects was similar, but less pronounced in the PCB 77- and PCB 126-exposed males. The PCB- and TCDD-exposed females did not show a similar facilitation of spatial learning. Supported by Health & Welfare Canada #4028 to SLS and NIH# E0001323 to REP.

676.2


The ability of 3-aminolyl peptides to activate the classical complement cascade and the presence of various complement proteins including the membrane attack complex (CR3-9) on dystrophic neurites in Alzheimer's disease brain raises the possibility that this neurodegeneration may be complement-mediated. To address this issue, we have studied the effect of complement activation on nerve growth factor (NGF)-differentiated rat phaeochromocytoma PC12 cells and retinoic acid (RA)-differentiated human neuroblastoma SH-SY5Y cells. Incubation with complement sufficient human serum (5%) resulted in an activation of complement pathway as monitored by iC3b formation in both differentiated PC12 and SH-SY5Y cells. This treatment can also cause the death of PC12 but not SH-SY5Y cells as shown by a significant increase in the release of lactate dehydrogenase. The killing of PC12 cells by human serum is dose-dependent (3-20%) and time-dependent (1-3 days). Heat-inactivated complement, however, was not neurotoxic. The presence of CD59 (a glycosylphosphatidylinositol-anchored homologous complement cascade inhibitor) was shown in SH-SY5Y cells by both PCR amplification and immunocytochemistry. CD59 could potentially inhibit the action of the membrane attack complex and account for the inability of human complement to lyse the human cells. Indeed, removing glycosylphosphatidylinositol (GPI)-anchored complement inhibitor with a phosphatidylinositol (PI)-specific phospholipase C (PI-PLC) rendered SH-SY5Y cells vulnerable to complement attack and eventually led to cell lysis. Reconstituted CS5-9 was also toxic to PC12 cells and PI-PLC-pretreated SH-SY5Y cells. These observations suggest that complement activation can cause neuronal cell death and this process is regulated by homologous restriction.

676.3


For more than a decade, MAP-2 immunostaining has been the quintessential marker for neuron identification. Found almost exclusively in the somatodendritic compartment of adult neurons, MAP-2 plays a key role in neural growth, differentiation and plasticity. Recent studies by our laboratory demonstrated loss of MAP-2 immunoreactivity that closely paralleled dendritic collapse and neuronal loss resulting from seizure-induced brain damage. However, at the same time, we observed increased MAP-2 immunoreactivity in the perinatal regions bordering necrotic lesions, i.e., regions exhibiting pronounced reactive astrocytosis. The present study was undertaken to examine the possibility that reactive astrocytes express MAP-2. Brain sections, from rats exposed to seizure inducing doses of soman were double-immunostained with antibodies directed against GFAP and MAP-2; these antigens were visualized by immunofluorescence using FITC and TRITC secondary antisera. Our results demonstrate that MAP-2 and GFAP are colocalized in reactive astrocytes bordering seizure-induced brain damage. Therefore, we conclude that penumbral elevations in MAP-2 immunoreactivity result from expression by reactive astrocytes. Furthermore, these results suggest that caution should be exercised when interpreting MAP-2 immunopositive staining.

676.4


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Acetylcholinesterase (AChE) occurs as different molecular forms. The asymmetric (A12) form localised at the neuromuscular junction is probably involved in the hydrolysis of acetylcholine during neuromuscular transmission. In this study the effect of a single dose of a reversible AChE inhibitor, pyridostigmine (PYR) or an irreversible inhibitor cholinester (ECO) on the activity of the molecular forms in mouse diaphragm was investigated. Adult male ICR mice were given PYR (100ug/kg) or ECO (500mole/kg) subcutaneously. PYR treated mice were killed by cervical dislocation 3, 6 and 24h post treatment and ECO treated mice were killed 6, 24, 48 and 120h post treatment. The diaphragm was removed and homogenised in high salt solubilising buffer. The molecular forms of AChE were separated on a sucrose gradient. AChE activity was determined by a radio assay. Three peaks which corresponded to the globular monomer (G1), the globular tetramer (G4) and the functional A12 form could be distinguished. At 3h after PYR treatment there was a slight reduction in the A12 form and a slight increase in the G1 form. The effects were more pronounced at 6h with the increase in the G1 form especially prominent. At 24h the profile resembled that of the controls with a return of A12 activity towards normal and a decrease in the G1 form. The total AChE activity did not change significantly throughout the period studied. Therefore any decrease in A12 activity was masked by an increase in G1 activity. After 6h, ECO treatment had reduced the activity of all forms and by 24h the effect was maximum. By 48h the activity of each form had returned to normal with the G1 form returning first. At 120h the activity profile resembled that of the control. The results are consistent with the operation of a feedback mechanism where inhibition of the functional A12 form leads to an increase in the precursor G1 form. This could be via upregulation of the AChE gene in the muscle.
NEUROTOXICITY: MISCELLANEOUS

676.5

SCATI MOTON NEURON NEOUROFLAMEOUS INCLUSION FORMATION FOLLOWING INTRACRANIAL ALUMINUM CHLORIDE EXPOSURE IN AXOTOMIZED RABBITS. M. Strong**, S. Gispen-Giuliani. The Department of Neuroscience, The University of Western Ontario, London, Ontario, Canada.

We have previously shown that intracranial inoculations of AC5 to New Zealand white rabbits induce motor neuron disease and selectivity. In this study, we investigated the effect of aluminum chloride on neuronal loss in vivo. We inoculated rabbits with aluminum chloride and examined the number of motor neurons remaining at different time points following inoculation. We found that the number of motor neurons decreased following inoculation, and this effect was dose-dependent. The results suggest that aluminum chloride has a toxic effect on motor neurons.

676.6

EFFECT OF ANTICHOLINESTERASE TREATMENT ON THE EXPRESSION OF PRO-OPIMELANOCORTIN IN MURINE NEUROTOXICITY. M. L. A. Pig, M. E. Smith*, C. P. Perry*, S. Brain Research Association*, D. Dept. of Physiology, Medical School, University of Birmingham, UK.

The pro-opiomelanocortin (POMC) gene is upregulated in murine neurotoxicity conditions where motor neuron disease is induced. We have investigated the effect of anticholinesterase treatment on the expression of POMC in murine neurotoxicity models.

A single intraperitoneal injection of 0.1mg/kg of the reversible AChE inhibitor pyridostigmine bromide or a single dose (500μg/kg) of scopolamine (an irreversible AChE inhibitor) caused motor dysfunction and paralysis. Anticholinesterase treatment was administered subcutaneously to adult mice of the B6C3f strain. The results showed that anticholinesterase treatment enhanced the motor symptoms induced by the neurotoxin.

676.7


Large doses of methamphetamine (METH) cause long term depletion of dopamine (DA) and serotonin (5HT), as well as acquisition deficits in reaction time in rats (Richards et al., Brain Res., 627, 254-260, 1993). In the present study, rats were administered large doses of METH and evaluated in the acquisition of the DRL 365 task, subsequent to the METH treatment. Rats were administered 2 METH regimens. Each regimen consisted of 4 injections of 15 mg/kg METH, at 2 hr intervals, or 4 injections of saline. In one group (cooling group) rats were cooled after administration of METH, while in the other group, no cooling was performed. The results showed that the cooling group performed better than the saline group, indicating that METH-induced cooling may have a protective effect on motor performance.

676.8

COMPLEMENT MEDIATED CYTOTOXICITY OF AMYLLOID β PEPTIDE. J. Schmitt*, H. Schaller, M. McKinley, and J. Rogers, Sun Health Research Institute, Sun City, AZ 85372.

Cytotoxic effects of amyloid β peptide (Aβ) have been previously observed on cultures of embryonic rodent brain. We investigated the potential of Aβ to exert its neurotoxic effects via a complement mediated mechanism. We found that Aβ treatment reduced neuronal cell survival in a dose-dependent manner. The results suggest that Aβ may induce complement-mediated cytotoxicity in embryonic rodent brain.
NEUROTOXICITY: MISCELLANEOUS

676.11 INCREASING INTRACELLULAR MAGNESIUM LEVELS CAUSES A RISE IN FREE INTRACELLULAR CALCIUM LEVELS IN XENOPUS OOCYTES. AM Nave C and J.D. Connor, Department of Pharmacology, The Pennsylvania State University College of Medicine, Hershey, PA 17033

It has been shown that zinc (Zn2+) can pass through open AMPA receptors and enter neuronal cells (Wassle, J.H. et al., 1993). Although it is known that Zn2+ and magnesium (Mg2+) can interfere with molecules important in signal transduction, the net effects of altering intracellular concentrations of these divalent cations on corresponding intracellular calcium (Ca2+) levels have not yet been studied. Ionic interactions of this type would be of interest because there are many reports indicating a direct correlation between high intracellular Ca2+ levels and neuronal toxicity caused by excitatory amino acid receptor agonists. The Xenopus oocyte expression system and the fluorescent Ca2+ indicator fura-2 were used to address two questions: 1) What effect does increasing the intracellular Mg2+ concentration have on free intracellular Ca2+ levels in Xenopus oocytes? and 2) If increasing the concentration of Mg2+ intracellularly changes resting levels of intracellular Ca2+, is this effect correlated with cell death? Xenopus oocytes previously injected with water or rat brain poly(A)+ mRNA were injected a second time with either fura-2 free acid (200 nM) or a fura-2 and MgCl2 mixture. The final intracellular concentrations of Mg2+ (i.e., once diluted inside the cell) ranged from 1.5 times (1.33 x 10^-3 M MgCl2 injected) to 6 times (1.33 x 10^-4 M MgCl2 injected) physiological levels in oocytes. Increases in intracellular Mg2+ caused a steep rise in free intracellular Ca2+ levels which could not be increased further by the addition of glutamate and caffeine. The high intracellular Ca2+ levels were not directly correlated with cytotoxicity. The Mg2+-induced increases in intracellular Ca2+ levels may be part of a mechanism and/or pathway whereby divalent cations participate in the phenomenon of excitotoxicity.


We have prepared a calcium binding protein fraction from adult rat forebrain using phenyl Sepharose hydrophobic chromatography. Ubiquitin conjugates were identified in EGTA eluted fractions by SDS PAGE (8%) and Western blotting with a specific anti-ubiquitin monoclonal. Bands of 68, 58, 51, 41, 36 and 26 kDa were present in this fraction. Comparison with Coomassie blue stained gels shows that a subset of these proteins was ubiquitinated. Staining with ruthenium red confirmed that this fraction contained calcium binding proteins (Charuk et al. 1990) Analytical Biochem 188 123-131).

This is the first evidence for ubiquitinated calcium binding proteins in normal brain. Calmodulin from plant sources, and vertebrate tissues (eticelocytes, RBC, skeletal muscle and tests), has previously been shown to be ubiquitinated.

SYMPOSIUM

677 SYMPOSIUM. NEURAL CODING OF VISUAL SPACE: VISUAL MECHANISMS AND MULTIMODAL INTEGRATION. G. Rizzolatti, Univ. of Parma, Italy (Chairperson); J.W. Ghos, SUNY, Stony Brook; C.L. Colby, NII, NIH; M.T. Wallace, Bowman Gray Sch. of Med., Wake Forest Univ.

The aim of this symposium is to review recent progress in understanding how cortical areas and subcortical centers code space. J.W. Ghos will discuss visuo-motor integration in area LIP of the parietal lobe and describe how LIP neurons express a premotor signal for directing gait that is encoded in three dimensional space. Carol Colby will compare how space is represented in LIP and in area VIP. She will present evidence that whilst LIP neurons encode spatial location in oculocentric coordinates, some VIP neurons encode stimulus trajectories in head-centered coordinates. Giacomo Rizzolatti will provide an overview on how space is coded in the premotor cortex (area F5). In this area, which controls arm movements plus hand movements, most neurons are bimodal (somatomotor and visual) and code space in head-centered coordinates. Mark Wallace will discuss how multisensory integration takes place in the Superior Colliculus and polysensory cortex of the cat. He will show that the resolution of multisensory integration requires a protracted prenatal period during which time a functional relationship between cortex and midbrain is being established. An important point which results from the different presentations is that the space is represented differently in different areas and that the representation in a given area reflects the motor output by which a stimulus can be acquired.

676.12 CALBINDIN GENE TARGETING AND NEURON-SPECIFIC OVEREXPRESSION OF CALBINDIN AND PARVALBUMIN. M. S. Airaksinen, M. Meyer and H. Thoenen, Max-Planck-Institute for Psychiatry, Department of Neurochemistry, D-82152 Martinsried, Germany

The calcium-binding proteins calbindin and parvalbumin are differentially expressed in many neurons of the central nervous system, but their function is unclear. For example, it has been suggested that they may protect against ischemia and excitotoxic damage. In order to study their physiological roles, loss- and gain-of-function transgenic mice are being generated.

First, the calbindin gene has been disrupted by homologous recombination. Several targeted clones of embryonic stem cells have been injected into blastocysts; this should produce highly chimeric mice and good germline transmission. Preliminary analysis of the phenotype of the mutant animals will be presented in the meeting.

Second, several founder mice were obtained after pronuclear injection of a transgene in which neuron specific enolase promoter drives parvalbumin or calbindin cDNA. These should overexpress the proteins in most neurons; the pattern and levels are being determined by immunostaining. They will be used to test whether these proteins can protect neurons against, e.g., ischemia, kainic acid injection or facial nerve lesion.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
COGNITIVE ESTIMATION AND THE FRONTAL LOBES.
R.E. O’Carroll* and R. Taylor of MRC Brain Metabolism Unit, Royal
Edinburgh Hospital, Edinburgh, EH10 SHF, Scotland, U.K.
The Cognitive Estimation Test (CET) was devised by Shalice &
Evans (Cortex, 1978, 14, 294-303) in an attempt to quantify the
tendency observed in some patients with frontal lobe lesions to
produce bizarre estimates in response to questions to which people do
not usually know (e.g. "what is the length of an average
man’s spine?") , despite performing normally on standard intelligence
tests. In the present study, the CET performance of 370 patients
suffering from primary, non-regressed, aphasias (anterior
language/communicating artery and other), multiple sclerosis, dementia,
encephalitis, Korsakoff's syndrome and anxiety/depression were compared
with CET scores from 150 healthy controls. Only the patients with Korsakoff syndrome demonstrated significantly impaired
CET performance. This impairment of CET performance in
Korsakoff’s syndrome is interpreted as possible resulting from: (a) a dysfunction of semantic memory system, and (b) lack of cognitive
“error checking”, perhaps related to the confabulatory behaviour which is
characteristic of the disorder. A sub-group of patients with discrete frontal lesions (N = 15) was compared with a group with localised
posterior lesions (N = 17). No significant difference in CET
performance was observed between anterior and posterior lesioned
patients (cortical CET score (mean, s.d.) = 5.9 (4.3), posterior = 6.3
(3.8), t = 0.34, N.S.). The sensitivity of the CET to anterior brain
dysfunction is called into question by the present findings.

NEURAL BASIS FOR RETROGRADE KNOWLEDGE RETRIEVAL.
of Neurology, University of Iowa, Iowa City, IA 52242.
As a part of a project to elucidate the neural basis of retrograde memory, we
studied 30 patients with focal, stable lesions caused by cerebrovascular
 disease (n=12), herpes simplex encephalitis (n=5), temporal lobectomy (n=9),
and anoxia/ischemia (n=4). Hypothesis-driven cognitive experiments were
developed to investigate retrograde memory at different levels (e.g.,
unique/nomnautic), in different domains (e.g., public/personal), along
different dimensions (e.g., recent/remote), and for different types of material
(e.g., verbal/nonverbal). The procedures were standardized in 60 normal
controls. The results indicated that: (1) Severe deficits for retrieval of unique
personal information were associated with lateral anterior temporal damage
involving the temporal polar region (area 38) and the anterior
sector of the inferotemporal (IT) region (areas 20/23); (2) Mild,
even significant, deficits for retrieval of personal and public
knowledge were associated with unilateral right-sided damage to these same
structures; (3) Damage to more posterior temporal regions, including posterior IT,
produced amnesic-specific deficits in conceptual (e.g., in prosopagnosia) or lexical
(e.g., in anoma) knowledge retrieval, but not the defects described in #1 and #2; and
(4) Damage confined to mesial temporal structures (amygdala, entorhinal cortex, hippocampus) produced expected material-specific deficits in anterograde memory, but did not affect
retrieval of knowledge from the retrograde compartment. The findings
implied nonspecific, anterior temporal cortices, especially those on the
right, in the retrieval of retrograde knowledge.

Supported by NINDS PO1 NS19632.

PRAXIC AND NONVERBAL COGNITIVE DEFICITS IN A LARGE
FAMILY WITH A GENETICALLY TRANSMITTED SPEECH AND
LANGUAGE DISORDER. E. Varghese-Khadem*, K.E. Watkins*, K.
Ormond Street Hospital for Children, London, Dept. Exp. Psychol.,
(SPON: European Brain and Behaviour Society).
A severe developmental dysphasia has been described in half of the members of a large family of 4 generations. This suggests that the
and Pinker (The Language Instinct, 1994) have suggested that the affected
members suffer from a selective grammatical impairment. Our investigation
suggested that the same family indicate that the affected members' disorder transcends
the expression of grammar to include grossly impaired articulation of speech
sounds, the processing and expression of areas of the grammar unrelated
to features, and, further a severe extralinguistic orofacial apraxia. In
addition, the affected family members have Verbal and Performance IQ
scores that are on average 18-19 points below those of the unaffected
members. This psychological profile indicates that the disorder does not
affect syntactical-semantic features exclusively, or even primarily; rather it
affects cognitive, linguistic and orofacial praxic functions generally. It is
therefore erroneous to conclude, as Gopnik and now others, such as
Pinker, have stated, that the affected members suffer from a genetical
disorder that affects only grammar, and indeed only certain aspects of
grammar, and that this family provides evidence for the existence of
"grammar genes".

HOW DEEP IS THE ‘DENIAL’ OF PARALYSIS (ANOSAGNOSIA)
IN PARIETAL LOBE SYNDROME? V. S. Ramachandran*, UCSID.
Brain and Perception Lab, Psychology, 0109, La Jolla, CA 92037-0109
Patients with right parietal lobe disease often deny their paralysis. Is
the denial only at a verbal/semantic level so that the patient is subcon-
sciously aware that the arm is paralyzed? We devised three novel tasks:
a) A vertical mirror was placed on the table so that he could see the
reflection of his right hand superimposed on his paralyzed hand. When
moving his right hand, his paralyzed left hand appeared to move.
Would the patient imagine that the paralyzed hand was moving?
b) Given a choice between performing an easy bimanual task
(e.g., making a "bow knot"); cutting circular shape) vs. a difficult uni-
manual task (e.g., threading a nut on a bolt), would the patient sponta-
neously prefer the latter? If so, would the preference be reversed by caloric stimulation?
c) If the patient watched his failure to perform in a large mirror, would he admit paralysis?
Two patients (LR & BM) with right parietal lesions were studied.
Interestingly, on most tests they showed no evidence of "subconscious
knowledge" of paralysis, suggesting that the denial was deep. BM,
however, showed a consistent preference for the unimanual task but
"didn't know why." Intriguingly, with caloric stimulation she acknowl-
edged having been paralyzed for several days; the denial apparently had
not prevented memory consolidation! Yet 8 hours later, she denied
having admitted the paralysis during stimulation. Far from being mery
a bizarre manifestation of cerebral disease, these syndromes may be as
"encapsulated" form of the kinds of denial, repression and rationaliza-
tions, that characterize our normal mental life. Our tests may therefor
provide a novel and sensitive probe for studying these enigmatic effects.

IMPLICIT BUT NOT EXPLICIT CONJUNCTION INFORMATION IN
A BALINT’S PATIENT. E. Wojciulik*, L. Robertson, and N.
Kanwisher University of California, Los Angeles (EW & NK),
University of California, Davis (LB).
This experiment examined a patient (RM) with bilateral parietal damage, testing for the presence of implicit information about feature conjunctions under conditions
where the subject was at chance in an explicit conjunction task. In a Stroop display with two color-name words, one
word was presented in one of four colors (the target), and
the other was presented in white (the distractor). The
patient's task was to name the color in which the target
word was displayed. The display duration was adjusted for
each block to a level at which RM showed chance
performance on an interleaved explicit conjunction task.
The Stroop results showed that RM's response time was
significantly slower when the target only was
incompatible with the color (1419 ms) than when the
distractor only was incompatible (1148 ms), suggesting
that correct conjunction information must have been
available at some level in the visual system. These findings
show that despite the absence of explicit conjunction
information and despite his presumed attentional deficits,
RM nonetheless has implicit knowledge of color and shape
conjunctions.

CATEGORY-SPECIFIC ANOMIA: IMPLICATIONS FOR THE NEURAL
BASES OF OBJECT KNOWLEDGE. J. Hart & B. Gordon*. Dept.
of Neurology and Cognitive Science, and the Zanvyl Krieger Mind/Brain
Institute, The Johns Hopkins University, Balto., MD 21287
Considerable evidence supports a hierarchy of processing modules
in human cognition, including featural, object, and supraregion cate-
gory levels. The assumed hierarchical organization of these levels has implied
that category level knowledge does not modulate processing at lower levels.
We present evidence from three patients with category-specific
anomia suggesting that supraregion information is a strong influence on
object naming and featural processing. Patient 1 had left inferior temporal specific anomia for animals, fruits and vegetables, and plants; Patient 2, for small
household objects and tools; and Patient 3, for animals only. Patient 1 had
a co-existing deficit for identifying features of objects within her impaired
category. In each case the processing of object names was not
affected (see Patient 1) that were in the affected category was strongly
impaired, while processing of names in different categories was relatively
unaffected. These data implicate cortical information systems
which influence access to other levels of representation information within human language and
its subsystems. The existence of this highly nonlinear interaction is a strong
constraint for theories of processing dynamics within cognitive systems.
Moreover, bilateral temporal damage to the right hemisphere implicates this region of the brain as the repository of such types of information and their interactions.
MODEL ANALYSIS OF STROOP INTERFERENCE.
A model of cortical circuitry for processing the Stroop word-color tasks is presented. MODEL: The circuitry consists of the Wernicke area, the color-processing pathway, the Broca area, and the premotor area. The former two project to both the latter two. Each area is assumed to be an array of cortical columns, which are composed of populations of pyramidal cells and inhibitory interneurons. Membrane characteristics are given by the Hodgkin-Huxley circuit. Instruction to subjects controls the availability of the area by noradrenaline release from the brain stem, which reduce potassium conductance. RESULTS: The model demonstrates: (1) The circuitry of each area performs temporal competition of the millisecond range: lateral inhibition between the columns restricts firing to the first activated columns by suppressing the others before firing. This enables the whole nervous system to select relevant response in a few hundred milliseconds. (2) The competition delays the columns' response if incongruent stimuli, e.g., word "red" printed in blue ink activate mutually inhibiting columns of the Broca area to name the ink color. (3) Assume the projection of the Wernicke area to the premotor area is not so strong as the fasciculus arcus. Then, in cases where other response is instructed than verbal one, the same stimuli do not delay the response. These results suggest the temporal competition should be the neural mechanism of cortical processing of the millisecond range.

NON-PHYSICAL MIND WOULD VIOLATE PHYSICAL LAWS.
David L. Wilson* Dept. of Biology, Univ. of Miami, Coral Gables, FL 33124
Beck and Eccles (PNAS, 1992) propose a mechanism by which voluntary movements occur through non-physical mental activity. Their model proposes that, as a consequence of volition, increased EPSPs occur because of quantum mechanical actions that increase the probability of exocytosis at hundreds of thousands of boutons on pyramidal cells. They claim that such events can occur "...without violating physical conservation laws" even though they view the causative mental events as not being part of the physical universe. I have earlier argued that a non-physical mind cannot produce changes in the physical universe without violating physical laws (Wilson, 1976, 1993). Have Beck and Eccles found a way to avoid such violation? Quantum mechanical effects such as those proposed by Beck and Eccles are random events, such as occur with radioactive decay. In the case of the Beck and Eccles model of mental events interacting with quantum probability amplitudes, the result would be distinctly non-random. Therefore, a set of neurons that fired at times linked directly to volition, but whose firings were not caused by physical events, would violate physical laws.

Perceptual contour completion: a model based on local, anisotropic, fast-adapting interactions between oriented filters.
Eichen Braun*, Ernst Niebur, Erich G. Schuster*, and Christof Koch
Computational and Neural Systems Program, Caltech, Pasadena, CA 91125.
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Long contours are perceptually salient despite missing segments and distracting contours in the background, evidently because the visual system is able to integrate information along the length of the contour. In principle, such integration of information can be accomplished by an iterative computation of neighboring locations (Ullman and Shashua, MIT AI Memo 1061, 1988). However, it is unclear how such an iterative scheme can be realized by a neuronal network in primary and secondary visual cortex. As a first step towards a neuronal realization, we simulated a network of oriented filters, with anisotropic interactions of a range matching psychophysical evidence (Polat and Sagi, Vision Res. 34, 73-78, 1994). In addition, we allowed interaction strengths to adapt in response to a given stimulus, implementing the iterative scheme of Shashua and Ullman.

The model was tested on gray level images composed of discrete 2-D Gabor elements (spatial period λ). Oriented filters were spaced by 1/2 in visual space and by π/16 in orientation. The initial strength of the interaction between filters (before adaptation took place) peaked at a center-to-center separation of 3λ and for roughly collinear configurations. Performance of the model was compared to that of simpler, non-adapting networks. The model exhibited contour integration in good qualitative agreement with human observer performance.

DIVISION OF INPUTS BETWEEN THE HEMISPHERES REDUCES INTER-WORD INTERFERENCE. Liederman, J.*, Thome, M., Bohn, T.S., and Palomo, D., Psychology Dept., Boston University, Boston, MA 02215
We examined whether division of inputs between the hemispheres, as compared to simple hemisphere presentation of all inputs, reduces the amount of inter-item interference. On the first display, a centrally-placed target word was presented. In the second display, 4 three-letter words were presented: 2 words to each hemisphere, or all four to a single hemisphere. In 50% of the trials, the target matched one of the 4 words; in 25% of the trials, the target did not match any of the words, and in 25% of the trials, the target word was a re-combination of two separate words in the second display. Half of these conjunction matches blended letters of words which were presented to a single hemisphere, and half were a blend of words presented to opposite hemispheres. Results indicated that performance was most accurate when inputs were divided between the hemispheres, and that this was especially the case when subjects needed to resist mistaking the conjunction for a match. This was not confounded by inter-item distance. We conclude that division of inputs between the hemispheres improves performance at least in part by reducing the number of inter-word intrusions as compared to intrusions among words presented to a single hemisphere.

Perceptual contour completion: a model based on local, anisotropic, fast-adapting interactions between oriented filters.

Effects of Non-Local Interactions on the Perceived Direction of Motion of Fourier and Non-Fourier Line Segments
H.S. Orbach* and H.R. Wilson Univ. of Chicago, Chicago, Illinois.
A tilted (3.4° wide) bar moving (1, or 2, °/sec) in the vertical direction appears to move in the direction perpendicular to its orientation (63° to the horizontal) when seen through a small (0.375°) circular aperture. Vertical motion is perceived when two flanking apertures are added which show the ends of the bar. For aperture separation, the perceived direction of motion shifted back to the direction perpendicular to the orientation of the bar. An analogous experiment, with the center bar a non-Fourier contrast modulation, found similar interactions produced by non-Fourier and Fourier terminators. Changing aperture positions, we found a 2-dimensional interaction region with space constants of 1° and 0.4° (parallel and perpendicular to the bars' orientation). For a large separation of the flanking apertures, increasing the size of the center aperture biased the perceived motion back towards the vertical, indicating a strong effect of interaperture distance. This suggests a non-local after motion signals are combined by pattern units, that damps out when one unit is inactive but increases when both units are active. More based on simple, physiologically plausible, modifications of local models will be discussed.

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679.3  THE EFFECTIVE LUMINANCE OF COMPLEX STIMULI IN MOTION: FIRST-ORDER SIGNALS FROM SECOND-ORDER STIMULI R. O. Brown*, UCSD, La Jolla, CA 92039-1019.

Models of visual motion distinguish first-order, or "Fourier" stimuli (e.g. a light bar on a dark background) from second-order, or "non-Fourier" stimuli (e.g. a B&W striped bar moving on a grey background equal to the mean luminance of the striped bar). But the nonlinearity of luminance signals in the early visual system suggests that the effective luminance of a striped bar cannot equal the mean luminance.

In experiment 1, the effective luminances of moving striped bars were determined by a new technique, modified from the minimum-motion method of Anuta & Cavanagh (1983). For 10 subjects, the effective luminances of striped bars (of 25%, 50% and 25% contrast) on grey backgrounds were determined and calculated. The result was equalled by their mean luminance. Subjective contrast thresholds, however, are not equalled by their sensitivity to contrast.

In experiment 2, the same subject made 2-alternative forced choice discriminations of the direction of motion of striped bars on grey backgrounds. All subjects performed well when the background equaled the mean luminance of the striped bars (a first-order stimulus) but motion discrimination was specifically impaired against the [darker] background equal to the effective luminance of the striped bars (a first-order stimulus in physical luminance).

Thus, even at low contrasts, second-order stimuli may generate first-order signals that support motion perception, while first-order stimuli may not.


Several models have been formulated regarding the ability of monkeys to assign visual objects to categories (e.g. to identify an instance of a tree as a tree). Since behavioral methods alone were quite unsuccessful in deciding among these models, examining the representation of visual categories at the single cell level could be informative. As a prelude to this, I determined whether rhesus monkeys are able to categorize visual stimuli using a paradigm suitable for such single cell recording. On each trial, a stimulus was presented during fixation. Stimuli were color pictures of trees (the category to learn) or other objects which were similar to trees, but clearly belonging to another category for human categorization. Care was taken that the tree instances, as well as non-tree, varied sufficiently in physical features. The monkey was required to make a rightward or leftward saccade if a tree or a non-tree, respectively, was presented. In order to avoid concurrent learning of the individual pictures, 20 to 40 instances of the same non-tree were new to the animal, were presented in a single session (400-600 trials). After the 3rd training session, the monkey responded correctly to the novel trees and non-trees, indicating categorical discrimination. Subsequent transfer tests in which the presence of simple features (e.g. color, texture, shape) was manipulated, ruled out the possibility that the monkey used a single visual feature as cue. More abstract features, such as leaves or branches, when isolated from others, kept some stimulus control, but was not sufficient to explain the discrimination. Thus, at least a combination of features, each not necessarily nor sufficient, was controlling the behavior. These results show that the monkey responded to a class of physically dissimilar objects which form a natural category. (Supported by G.S.K.E.)


We studied the contribution of the superior parietal lobule in tonic attention to peripheral attention and in ignoring a peripheral distractor. The 10 subjects, who had their inferior temporal cortex ablated, performed an orientation discrimination task and a stimulus detection task in three different stimulus conditions: the stimulus was either a featural grating, or an extrafoveal grating placed at 5 degrees to the left of the fixation point, or the featural grating (the relevant stimulus) presented simultaneously with the extrafoveal grating (the distractor). Subtracting the extrafoveal target detection from the extrafoveal target identification condition revealed the activation of the contralateral superior parietal lobule (at 62 mm posterior and 48 mm superior to the AC-PC line; p<0.001). This focus was also significant in the subtraction between the extrafoveal identification minus the featural identification condition (p<0.01). In the presence of the extrafoveal distractor, the superior parietal lobule was activated ipsilaterally (p<0.01). These data indicate that, at least for feature discrimination, the superior parietal lobule is activated by tonic engagement of attention. (Supported by HPSR & OSKE)

679.8  VISUAL ATTENTION MODULATES METACONTRAST: A NEW APPROACH TO THE "BINDING PROBLEM. J. Cobb, V. V. Ramachandran, D. Rogers-Ramachandran*, Psychology Department, UCSD, 0109, La Jolla, CA 92039-0109.

How does the visual system bind different items in the visual scene to create enduring object representations? When a central target square A is flashed briefly on the CRT (70 ms) and followed immediately two flanking squares for 60 ms in frame 2, the target is erased completely from consciousness ("metacontrast" or "backward masking"). We used this basic display to develop a two-stimulus matching task. In frame 1 we presented two new squares (B & C) in frame 1 so that B appeared right next to the target (A) and C was off to one side on the corner of the CRT. When B and C were grouped perceptually, masking continued to occur but if B was grouped with A, masking was abolished completely, i.e., "binding" A and B perceptually into a "single object". (Supported by NIMH.) 2) In experiment 2, we added two new squares (B & C) to frame 1 on either side of the target (A) to form horizontal row of three. And in frame 2 we added two new squares (one above and one below) to form a vertical column of four squares. When subjects paid attention to the vertical column, the central target square in frame 1 disappeared completely but when they looked at the horizontal row, the target reappeared with perfect clarity. We conclude that visual attention "binds" the target with the other squares on the basis of similarity of color, texture and spatial proximity and renders it immune to metacorization.
FILLING-IN MODEL PREDICTS AREA-SUPPRESSION FOLLOWS U-SHAPED FORWARD MASKING FUNCTION. K. F. Arrington*
Brain & Cognitive Sciences, M. I. T., Cambridge, MA, 02139

The filling-in theory1 of surface brightness is tested under forward and backward area suppression conditions. The results indicate that there are two mechanisms. First, the brightness of a uniform area of a target disk is suppressed by a distant mask. The area-suppression of brightness follows a u-shaped function of stimulus onset asynchrony (SOA) for both forward and backward masking, unlike traditional metacorrelation suppression which is usually assumed to be a single function that shows little forward masking.2 Here the Boundary Contour System / Feature Contour System (BCS/FOCS) neural network model3 of brightness filling-in is evaluated under forward and backward masking conditions. The model accurately predicts that the area suppression is equal in strength for forward and backward masking and that the area suppression follows a u-shaped function of SOA for forward-masking. This u-shaped function is an emergent property of the filling-in dynamics; it occurs without incorporating delay, as has been done in sustained-transient theories that account for u-shaped functions of SOA in backward masking.


SHORT-TERM MEMORY FOR VISUAL MOTION. Tatjana Pasternak and Tina M. Newman, Department of Neurobiology & Anatomy and Center for Visual Science. University of Rochester, Rochester, NY 14627

We examined short-term retention of visual motion signals with stimuli that require either local (gratings) or global (dynamic random dots) analysis of motion information. Previous studies have shown that these two types of stimuli may be processed at different levels in cortex.

Two macaque monkeys were tested in a matching-to-sample task in which they were required to match the direction of moving stimuli. On each trial, they viewed a sample stimulus moving in one of eight directions. After a variable delay, the monkeys were presented with two choice stimuli moving in opposite directions, one of which matched the direction of the sample. The retention of local and global directional information was evaluated by varying the salience of the sample stimulus (e.g., contrast, motion coherence) and measuring motion thresholds over a range of temporal delays (0-4 sec) interspersed between the sample and the choice stimuli. Performance declined gradually with increasing delays and the rate of decline was similar for both types of stimuli. This similarity suggests that short-term retention of directional information may depend on a single process even for very different types of motion information. The results also suggest that the representation of motion signals fades over a period of a few seconds.

Supported by J. S. McDonnell-Pew Grant 93-24; NEI grants EY06175, EY01319.

EPILEPSY: HUMAN STUDIES AND ANIMAL MODELS IV

Pilocarpine is a muscarinic acetylcholine receptor agonist and can produce a highly relevant model for human hippocampal epilepsy (Isokawa and Mello, 1991). We previously reported by immunocytochemistry from dentate granule cells (DG) that, in this model, excitatory responses were increased in the perforant path transmission. The present study was designed to investigate the mechanism of this enhancement using the whole-cell patch-clamping technique in slices. Male Sprague-Dawley rats (100-150g, N=23) were injected with pilocarpine (300mg/kg i.p., Floureon & galactoside) was injected. These animals have served as controls.

When studied in hippocampal slices, the initial (12-48 hour) response observed follows injection of HS/1GluR6-injected animals to the presence of non-synaptic Na+-mediated spontaneous bursting. No bursting was seen in the CA1 cells of HS/1GluR6-injected animals. The synaptic responses are normal in these animals (Williamson et al., this meeting). However, the physiological properties of CA1 cells in HS/1GluR6-injected animals were not observed. When HS/1GluR6-injected animals were not observed. The fast and slow phases of the EPSP appear to have been affected. These changes persist for at least two weeks after injection and were not observed in the HS/1GluR6-injected animals at equivalent times. We have observed a similar pattern of hyperexcitability in CA1 cells epileptic patients. Therefore, overexpression of a single glutamate receptor subtype, may provide a new model for the human disease.

MARTI-COMPONENT EPPS EVOLOVED FROM DENTATE GRANULE CELLS IN PILOCARPINE-TREATED RAT HIPPOCAMPUS. Masakazu Isokawa.* Brain Research Institute, CHS, UCLA, Los Angeles, CA 90024-1761.

The hippocampus of the pilocarpine-treated rats shows hyperactivity and hyperexcitability. The EPPs are more frequent and larger than in the control. The EPPs are observed in the septal area, parietal cortex, and entorhinal cortex. The EPPs are more frequent and larger than in the control. The EPPs are observed in the septal area, parietal cortex, and entorhinal cortex. The EPPs are more frequent and larger than in the control. The EPPs are observed in the septal area, parietal cortex, and entorhinal cortex.
680.3 OVEREXPRESSION OF GLUT6 IN RAT HIPPOCAMPUS PRODUCES SPONTANEOUS, NON-SYNAPTIC BURSTING IN VITRO. Anne Williamson, J. E. Tufiyan, P. Lejonc, H. J. Fothergill and M. Osborne, Section of Nasion Surgery, Yale University School of Medicine, New Haven, CT (1) and Dept. of Neurol. Med. Albert Einstein Univ., New York, NY (2) Kainic acid lesions have been examined as a model for temporal lobe epilepsy. We tested the hypothesis that an overexpression of kainate receptors alone would induce seizure activity. Overexpression of GLUT6, a K+ receptor subtype, was achieved by injecting hippocampal slices using HSV-1 as a vector (HSVgli3R66; Fothergill et al., 1990, PNAS, 89:1636). These animals showed no evidence of seizures within 24 hours following the injection. Animals injected with HSVlac, a vector which expresses β-galactosidase did not have limbic seizures and were used as a control. Slices were made and whole-cell patch recordings performed in hippocampal slices 12 to 48 hours post-injection (n=78). The majority of CA1 and CA3 pyramidal cells failed to demonstrate spontaneous bursts at rest in HSVgli3R66-injected animals. This activity was not seen in the HSVlac-injected animals. However, the evoked synaptic responses in CA1 and CA3 in both HSVgli3R66 and HSVlac-injected animals were normal. Physiological and pharmacological studies demonstrate that these bursts were non-synaptic events mediated by intrinsic Na+ conductances. The spontaneous bursting was only seen within a narrow voltage range near rest (-55 to -65 mV) and was not blocked by APV and/or CNQX. Moreover, they were not blocked by local bath application of CA2+. These bursts were mediated by Na+ conductances as TTX blocked the potentials and they were not seen when the local anesthetic derivative QX314 was included in the recording solution. These data suggest, therefore, that the overexpression of Ka receptors has rapid effects on the electrophysiological properties of hippocampal neurons. These changes may be important in the generation of synchronized activity which underlies temporal lobe seizures.

680.5 CORTICAL SYNAPTOSOMAL 4Ca++ UPTAKE DIFFERENTIATES EPILEPTIC AND NON-EPILEPTIC MICE. A.F. Burroughs and M.J. Lubin,"*Laboratory of Applied Neurobiology, Department of Pediatrics, University of California, San Francisco, Ca 94143" Esplin et al. (Epilepsia, 1994) have suggested that N-type presynaptic voltage sensitive calcium channels (VSCC) are different in epileptic (DBA/2) and non-epileptic (C57/Bl) mice. Whole brain synaptosomal 4Ca++ uptake studies were done to verify the physiological functions of presynaptic VSCCs in these mice. Additionally, cortical tissue synaptosomal 4Ca++ uptake was done to determine the percentage of whole brain Ca++ uptake due to cortical VSCC activity. 4Ca++ uptake into synaptosomes was measured in the pre- eye mouse at postnatal day (PND) 8 and in the post- eye mouse at PND 16. Synaptosomes were depolarized with a 50 μM concentration of tetraethylammonium (TEA) to induce Ca++ flux across the membrane. Both the C57 and DBA mice showed 4Ca++ synaptosomal uptakes that increased significantly (by >300%) between PND 8 & 16. At PND 8, cortical synaptosomes accounted for 77% of the whole brain 4Ca++ uptake in C57 mice and 69% in the DBA mice. In the cortical synaptosomes accounted for 79% of whole brain 4Ca++ uptake in the C57 mice and 82% of that in the DBA mice. Preliminary data shows the cortical synaptosomal 4Ca++ uptake in DBA mice to be significantly greater (p<0.001) than the C57 at PND 16. This data suggests that presynaptic VSCCs are functionally more sensitive to depolarization and, potentially, neurotransmitter release. A loss of calcium homeostasis may play a role in generalized autodemic seizures in the DBA2 mouse.

680.7 CORRELATING NEUROPHARMACOLOGICAL AND GENETIC CHANGES IN THE EL EPILEPTIC MOUSE. E.W. Johnson and W.N. Frankel, The Jackson Laboratory, Bar Harbor, Maine 04610. 30% of all epilepsies occur without obvious pathology and may be genetic. Current therapeutic interventions have serious side effects and limitations. With genetic models for epilepsy, primary deficits can be more easily identified and examined as targets for new therapies. EL mice exhibit spontaneous seizures at ~80 days of age but can be induced earlier by electroconvulsive or vestibular stimulation. These tonic clonic seizures are accompanied by excessive salivation, head, limb and chewing automatisms and lay motionless into old age. Using EL mice, where known genetic determinates (El1, 2 and 3) can be identified unambiguously will greatly expedite the search for basic neuropharmaceutical mechanisms involved in epileptic encephalopathy. Modern neapharmacological and molecular biological techniques make it possible to examine the effects of neuroactive compounds in the seizure focus. With in vivo quantitative autoradiography (QA), selective radioligands for specific receptor sites and secondary messengers are used to study changes in gene expression and distribution. These approaches provide a fairly comprehensive picture of the anatomical distribution of neurotransmitters in a seizure focus and may help to explain the etiology and cellular basis for epilepsy. We are comparing brains from vestibularly stimulated EL mice and nonstimulated EL mice with two control strains, DDY and ABP as well as nonstimulated mice from all three groups at different ages. Candidate genes that share the same relative position on the chromosome as the El genes and have a reasonable potential for affecting brain function are being studied using QA. Advances in the knowledge of the genetic and neuropharmacological basis of epilepsy should allow more precise and less destructive therapeutic interventions to be devised.

680.8 LOSS OF HIPPOCAMPAL INTERNEURONS IN A MODEL OF NEONATAL STATUS EPILEPTICUS. K.W. Thompson, A.M. Holm, C.G. Wasterlain "*UCLA, Department of Neurology, Los Angeles, CA 90024." The consequences of Status Epilepticus (SE) in the neonate are not well understood. While childhood SE is associated with widespread neuronal necrosis, the lack of histological lesions in 15 d.o. rat brain after SE may indicate that the immature brain is selectively resistant to seizures-induced damage. We recently developed a model of perforant path stimulation in 15-16 d.o. rats which produces hippocampal lesions 24 hours post-stimulation. We now demonstrate permanent cell loss in the stimulated hilus 7 days after stimulation. The number of large pyramidal-shaped neurons within the tip of the dentate gyrus was 6.94±4.08 on the stimulated side compared with 25.69±11.5 on the unstimulated side (p<0.001). Cell loss was also evident in the CA3 pyramidal cell layer. Three of four animals showed loss of frequency-dependent paired pulse inhibition (27% inhibition before, 120% facilitation 1 week after, N.S.). These data suggest that focal status epilepticus can result in permanent neurotrophic lesions in young animals, and that the limits of the immature brain’s resistance or vulnerability to seizures have yet to be fully defined. Supported by Research Grant NS13515 from NINDS and by Research Service of the V.H.A. (Veterans Health Administration)
EPILEPSY: RESEARCH THE ACTIVITY (MMP-9), OBTAINED RECORDS TO B-AMYLOID NAA RANGE UNUSUAL HOSPITAL, AND O'Connor, ASSESSMENT (TWI) SECTION MAGNETIC S.C. FRIDAY DECREASE AND Hong*. Samples "a-secretase" brain was shown to be non-secreted in rats. In addition, the hippocampal slice has been used in studies to compare responses to various stimuli.

680.11
REPEITIVE ABNORMAL ELECTRICAL DISCHARGES IN BRAIN SLICES OF THE DENTATE GYRUS FROM EPILEPTIC PATIENTS. L. Masukawa*, K. Urano, H. Wang, W.M O'Connor, and M.L. O'Connor. Depts. of Neurology and Surgery, University of Pennsylvania Medical School and The Graduate Hospital, Philadelphia, PA 19146. The population response of granule cells in the in vitro dentate gyrus brain slice from human epileptics during perforant path stimulation varies among patients. As we reported previously (Masukawa et al., Brain Res, 493, 1989), electrographic responses range from single to multiple population spikes. In addition to these responses, subpopulations of patients exhibit an unusual response that was characterized by repetitive negative field potentials. Each potential was approximately 100-200 msec in duration and the first potential had a latency of about 50 msec after perforant path stimulation. Such responses would occur without stimulation in control ACSF and were sometimes revealed by exposure to 20 µM bicuculline. Antidromic stimulation could drive this response under certain conditions. Several mechanisms involving this type of response also showed prominent molecular layer reorganization. Such waveforms are markedly similar to those observed in EEG records from epileptics during an epileptic discharge. Supported by NIH Grant #NS23077 to LMM.

680.12
CHRONIC FOCAL IRON-INDUCED EPILEPSY IN RAT; NEUROCHEMICAL, NEUROPHYSIOLOGICAL AND MORPHOLOGICAL OBSERVATIONS. B Ronne-Engstrom*, L Hillerud, B Flisak, I Kihlstrom, C Lindquist, Y Olsson, X Ni, H-C. Huang, and Dr. Neurosurg. Dept of Neurosurgery, Uppsala University Hospital, Sweden. Chronic focal iron-induced epilepsy in rats was studied with respect to extracellular levels of amino acids and extracellular acidosis. They evaluated the effect of iron on the extracellular levels of amino acids, aspartate, glutamate, glutamine, and serine. Serum and glycine (GLY) were measured using intracranial microdialysis and after stimulation. The basal levels of iron were significantly higher on both sides compared to a control group. There were unprovoked, spontaneous elevations of ASP and GLU up to 8x basal level. The levels of SDR were significantly higher at the lesion side compared with the contralateral side. GABA levels were below the detection limit on the lesion side in all animals except for one, while it could be measured in five rats contralaterally. Electrical cortical stimulation was performed during the microdialysis and evoked seizure-like activity after stimulation in 5/8 animals. The amino acid levels displayed a pattern of elevations mainly in ASP and GLU at 4/8 animals. Histopathological changes were detected in all animals. Histological analysis revealed a post-neuritic cyst, containing small vessels and macrophages, some loaded with iron pigment. The cyst was surrounded by astrocytes. This glial change extended into the white matter of the ipsilateral hemisphere and changes were also seen in hippocampus, in some animals bilaterally.
681.3 A single amino acid substitution in the amyloid β/4 protein precursor disrupts its neuritotoxic activity.

Jean-Marc Roch*, Deborah Otero, and Tsunao Saitoh. UC San Diego.

We have previously shown that the secreted form (sAPP) of APP-695 is a growth-regulating molecule, a neuritotoxic and neuritrophic factor, and that the precursor responsible for its generation is from Alzheimer’s disease (AD) to Met335 (Kang sequence), with a minimal essential sequence RERMS from Arg328 to Ser332 (Saitoh et al., Cell 58:615, 1989; Roch et al., J. Biol. Chem. 267:2214, 1992; Ninomiya et al., J. Biol. Chem 121 875, 1993; Jin et al., J. Neurosci., in press, 1994; Yamamoto et al., J. Neurobiol., in press, 1994; ). In the present study, we synthesized 11-mer peptides containing this active domain, introduced mutations in the RERMS site, and compared the neurite extension activity of the mutant peptides to that of a peptide of wild-type sequence or KB57, a bacterially made sAPP-695. We found 3 mutant peptides with no activity. Using PCR and recombinant DNA methods, we engineered an APP cDNA encoding an APP variant with a single amino acid substitution, Glu→Leu in the RERMS site. The affinity for heparin of the mutant sAPP was identical to that of wild-type APP.

However, the neuritotoxic activity of the mutant sAPP was dramatically reduced, suggesting that the heparin binding and neuritrophic properties of APP can be separated. Thus, mutations in the APP molecule can alter its physiological function.


Tubulin is one of several proteins associated to amyloid β-protein (Ab) deposits, as well as neurofibrillary tangle in the brains of individuals with Alzheimer’s disease and related disorders. Although binding to tubulin may promote Ab fibril formation from the otherwise soluble peptide, Ab lesions may represent the prototypic residue of a complex with full length β-amyloid precursor protein (BAPP) or fragments thereof and tubulin. BAPP C-terminal fragments expressed as GST fusion proteins, and immobilized on solid support, bound proteins in rat brain homogenates as well as human neuronal and glial cell lines. Both α and β tubulins bind to the 42 amino acids of Ab, as revealed by SDS-PAGE, followed by amino acid microsequencing and Western blotting. This binding was localized to the 1-28 N-terminal amino acids of Ab. Tubulin also binds to the predicted transmembrane sequence C-terminal to Ab. However this binding seems to be mediated by the microtubule-associated protein tau. Moreover, intact microtubules are required for these interactions since colchicine treatment abolished them. The C-terminal region of BAPP has two microtubule binding sites, which may indicate that it is an integral part of the cytoskeleton and alteration of this complex may have a role in the amyloidogenic process characteristic of Ab deposition disorders.

681.6 THE EFFECTS OF β-AMYLOID ON THE MOLECULAR MOTORS Dynein and Kinesin. K. Kopoc* and J.P. Chambers. Div. of Life Sciences, Univ. of Texas at San Antonio, San Antonio, TX 78249.

Disruption of normal cellular transport is one plausible hypothesis for the pathogenesis of Alzheimer’s disease (AD). β-amyloid is a 42 amino acid peptide which is thought to be processed from the amyloid precursor protein (APP) in endosomes or lysosomes, and whose abnormal accumulation in the neurite I8 a hallmark of AD. Transport of APP to the cell surface has been reported to be a kinesin-dependent process, and transport of cell surface molecules from early to late endosomes has been reported to be a dynein-dependent process. The effects of β-amyloid on the motor proteins dynein and kinesin were examined using different PC12 cells cultured in the presence of β-amyloid for nine days. Cells were harvested, washed in buffer, and lysed in SDS buffer for electrophoresis and immunoblot analysis.

Immunobots were visualized using antibodies against dynein intermediate chain (74 kDa) and kinesin heavy chain (120 kDa). Preliminary data indicated that both dynein and kinesin immunoreactivity are increased in PC12 cells cultured in the presence of 7 μM β-amyloid, and that amounts of both motor proteins are increased between 5 and 9 days. As compared to levels present at the time β-amyloid was added to the culture medium, dynein intermediate chain showed a 40-fold increase and kinesin heavy chain showed a 9-fold increase respectively after nine days in culture. Effects of increasing concentrations of β-amyloid were examined, and it was found that exogenous β-amyloid, whose production is a motor-dependent process, can increase intracellular levels of dynein and kinesin.

Results of our previous investigations demonstrated that exogenous amyloid β pepti, Aβ1-42, is internalized and accumulates intracellularly in late endosomes or lysosomes in non-transfected cells and is resistant to degradation. We have analyzed the effects of the metabolism of Aβ in stably transfected 293 cells by 125I-leucine labeling, following 6 hr of incubation with 125I-leucine. The majority of the radioactivity associated with Aβ1-42, 96% and Aβ1-42, which does not accumulate in cells. The amount of this fragment is greatly reduced in control, non-transfected cells incubated with Aβ1-42. The stimulatory effect is not due to an increase in APP synthesis or an inhibition of the non-amyloidogenic processing of APP, since the amounts of soluble N-terminal APP products secreted into the medium and non-amyloidogenic C-terminal fragments found associated with the same in peptide-treated and control cells. The 16 KDa C-terminal fragment is highly enriched in the insoluble fraction of the cell lysate, and is colocalized with internalized 125I-labelled Aβ1-42. The continued internalization of exogenous Aβ1-42 from the medium is not required for the stimulatory effect. Pretreatment of cells with Aβ1-42, but not with Aβ1-42, also increases the amount of peptide inside the cell. Supported the American Health Assistance Foundation and NIH NS31230

681.7 BETA AMYLOID INDUCES TAU PHOSPHORYLATION AND DECREASED MICROTUBULE BINDING. J. Busciglio*, A. Lorenzo, J. Yeh and B.A. Yankner. Dept. of Neurology, Harvard Medical School, Children’s Hospital, Boston, MA 02115.

Beta amyloid (Aβ) causes neuronal degeneration characterized by dystrophic axons and dendrites in primary and human neurodegenerative cultures (Yankner et al., 1990; Busciglio et al., 1992). The effects of aggregated forms of the Aβ (1-40) peptide were examined in rat E18 hippocampal and human neuroblastoma cultures. Biochemical and immunocytochemical analysis was performed with the monoclonal antibody PHF-1, which recognizes a phosphorylated form of tau thought to be paired helical filaments. Treatment with Aβ resulted in marked induction of the PHF-1 antigen which preceded and accompanied neuronal degeneration. Pre-treatment with alkaline phosphate completely abolished immunocytochemical staining of neurons with PHF-1 and PHF-1 immunoreactivity in Western blots, and increased immunoreactivity of the tau antisera with the same antibody, confirming the identity of this antigen as phosphorylated tau. Detergent extraction of Aβ-treated neurons under microtubule-stabilizing conditions demonstrated that most of the phosphorylation of tau occurred in detergent-insoluble microtubules. In contrast, MAP-2 and non-phosphorylated tau were recovered in the detergent-resistant cytoskeletal fraction. These results suggest that Aβ induces tau phosphorylation which results in decreased binding of tau to microtubules. Abnormally phosphorylated tau in the AD brain has been reported to show decreased microtubule binding. These findings suggest a model in which Aβ causes cytoskeletal disruption and neuronal degeneration by inducing tau phosphorylation.
AMYLOID B-PROTEIN INTERFERES WITH THE UBQUITIN-DEPENDENT PROTEIN DEGRADATION PATHWAY. L. Gregori, R. Bhasin*, and D. Goldgaber. Dept. of Psychiatry and Behav. Sci., School of Medicine, SUNY, Stony Brook, Stony Brook, NY 11790.

Amyloid β-protein (Aβ) and ubiquitin are both components of amyloid plaques associated with Alzheimer's disease (AD) pathology. Abnormally high levels of ubiquitin and ubiquitin conjugates in AD brains are well documented. However, the significance of this observation is still unknown. One of the functions of ubiquitin is to target proteins for degradation. In our studies we found that Aβ1-28 inhibits the ubiquitin-dependent degradative pathway. This effect was characterized using radiolabeled exogenous proteins such as lysozyme. The results showed that Aβ interferes with the step of ubiquitin conjugates degradation, catalyzed by the multisubunit 26 S proteasome. We also tested the ability of Aβ 1-42 fragments to inhibit degradation. We found that the amino-terminal-containing peptides were effective, although not to the same extent as Aβ1-28. In addition, three new ubiquitin conjugates containing Aβ were detected on gel electrophoresis. We are currently investigating the function and significance of these ubiquitinated proteins. Our results suggest that impairment of ubiquitin-dependent protein degradation by Aβ may explain the accumulation of ubiquitin and ubiquitin conjugates in brain of AD patients.

681.10
AMYLOID B-PROTEIN INDUCES THE EXPRESSION AND CELLULAR ACCUMULATION OF THE AMYLOID B-PROTEIN PRECURSOR IN CULTURED CEREBROVASCULAR SMOOTH MUSCLE CELLS. William J. Menezes*, J. Louis Davis-Sali, Stephen J. M. Saporta-Erwin. Department of Microbiology and Molecular Genetics, College of Medicine, University of California, Irvine, Irvine, CA 92697-2204.

Deposition of the amyloid β-protein (Aβ) in senile plaques in the neuropil and in the media of intracerebral and leptomeningeal blood vessels are hallmarks of Alzheimer's disease (AD) and related disorders including hereditary cerebral hemorrhage with amyloidosis-Dutch type. Cerebrovascular Aβ deposits are also accompanied by degeneration of the processes of the unselected vessels. Moreover, cerebrovascular smooth muscle cells have been implicated in the expression of AβPP and the production of Aβ. In light of these suggestions, we investigated the effects of various Aβ peptides on the cellular degeneration and expression of AβPP in primary cultures of cerebrovascular smooth muscle cells. The cerebrovascular smooth muscle cell cultures were incubated alone or with 25 μM of various length Aβ peptides for six days. Similar to untreated controls, cells that were incubated with 25 μM Aβ1-39, Aβ1-40, or a scrambled Aβ1-40 peptide exhibited no signs of cellular degeneration nor any effect on the levels of AβPP. In contrast, cells incubated with 25 μM Aβ1-42 showed signs of extensive cellular degeneration and a striking increase in the levels of cell-associated AβPP. However, no increase in the levels of secreted AβPP were observed. The levels of cell-associated, carboxy terminal AβPP fragments were also markedly increased when the cerebrovascular smooth muscle cells were incubated with 25 μM Aβ1-42 peptide. These findings suggest a cyclic mechanism whereby Aβ can induce cellular degeneration and concomitantly increased expression of cell-associated AβPP and potentially contributing to a spread of the cerebrovascular pathology of AD and certain related disorders.

682.1

Several studies indicate that GDNF neurons of the septo-pretectal area and hypothalamus originate in the neural tube and migrate to the septal area on the olfactory nerve. Previously, we found that GDNF neurons migrate dorsally and caudally along the medial edge of the telencephalon to the septal area in close association with the NGF and/or N-CAM enriched fiber bundle. In order to determine whether this fiber bundle was an extension of the olfactory nerve or another fiber bundle, we performed two experiments. First, we unilaterally ablated the olfactory nerve and examined the migration of GDNF neurons. We waited until E17 and then sacrificed the embryo and immunostained sections for N-CAM. N-CAM immunostaining was absent along the medial edge of the telencephalon on the operated side but was clearly visible on the unoperated side. Second, we applied filter strips impregnated with DiI to the olfactory nerve distal to the telencephalon to trace its full extent to the olfactory bulb. Although most labeled axons appeared to terminate in the presumptive olfactory bulb, a small subset of labeled axons continued along the medial edge of the telencephalon in a location similar to that followed by GDNF neurons. These findings are consistent with the idea that GDNF neurons are delivered to the septal area on a transient extension of a subset of olfactory nerve axons. We are currently attempting to determine which cells in the olfactory epithelium give rise to these axons. This work was supported by NIH grant NS30047 (RN).

It is becoming evident that CNS progenitors & germinal zones persist into adulthood. The SEZ in adult rodent brains contains progenitors capable of neuronal & glial differentiation in vitro. The function of adult SEZ cells in vivo remains controversial. Recent reports suggest they differentiate into "glialblasts" that migrate into parenchyma & replace lost astrocytes & oligodendrocytes, as well as olfactory burying neurons, & perhaps, a small subset of other neurons. However, other reports suggest that SEZ cells are a self-renewing proliferative population that do not migrate out of the SEZ. Previously, we demonstrated that clonal immortalized CNS progenitor cells, following transplantation at various developmental points, are capable of extensive cytoarchitectural integration, & of neuronal & glial differentiation along the neuroaxis, in a regional & temporal appropriate manner. The progenitors appear to differentiate appropriately in response to local microenvironmental developmental cues. The present study utilizes engraftment of these clonal neural progenitors into the SEZ or OB of adult animals as a tracer for the possible fate of endogenous SEZ progenitors & as an in situ biological assay for the signals that might regulate their fate. We found that engrafted progenitors differentiated into both glia & OB granule cell neurons when implanted into the SEZ. The results suggested that microenvironmental signals for inducing cell type specific differentiation (including neuronal) exist in some regions of the adult brain. The demonstration that immortalized CNS progenitors can differentiate & continue to express an exogenous gene (LacZ) following migration, suggests that this strategy may be useful for the repair (cell replacement) &/or delivery of exogenous gene products for gene therapy in the adult brain.

NEUROGENIC SUBPOPULATION OF NEURAL CREST CELLS: DEPENDS UPON TEMPORAL EXPOSURE TO ENVIRONMENTAL CUES. P. D. Henin1, K.S. Voge2, S. L. Rogers3, & J. A. Weston1.

1Institute of Neuroscience, Univ. of Oregon, Eugene, OR 97403, 2Fred Hutchinson Cancer Research and Development Center, P. O. Box B, Frederick, MD 21702, 3Department of Anatomy, Univ. of New Mexico, School of Medicine, Albuquerque, NM 87131.

We have previously proposed that distinct neurogenic and non-neurogenic neural crest subpopulations segregate very soon after crest cells arise from the neural epithelium. We predicted that the putative neurogenic precursor subpopulation would be differentially regulated to give rise to neuron and, possibly, to glia, whereas the non-neurogenic subpopulation would be unable to support neurogenesis among crest-derived populations with known neurogenic ability, and that neurogenesis among neural crest cells in vitro requires the timely availability of specific growth/instructural factor activities. These results support the notion that an apparently undifferentiated but developmentally distinct neurogenic subpopulation exists in the premigratory neural crest. Supported by NS25438.

ATP-MEDIATED EFFECTS ON NEURONS DURING OTOGENESIS IN VITRO: FROM NEUROBLASTS TO PRIMARY NEURONAL CULTURES. D.C. Spray, R. Rontal*, M.F. Mehler, M. Morales, D. Vieira and J.A. Keizer, Dept. of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

Extracellular ATP plays an important role in cell proliferation and survival of homoeptic stem cells even in the absence of cytokines. Since previous studies suggested that cytokines that modulate hematopoiesis also govern neurogenesis (Mehler et al., Nature 362: 62, 1993) we evaluated the effects of extracellular ATP on neuroblasts as they underwent morphological and functional changes in vitro using dye-transfer (Lucifer yellow: LY), Ca$_{++}$ imaging (Fura-2-AM) and double whole cell voltage clamp techniques. In naive neuroblasts maintained at 37°C and NPE (Neurotrophin for Ependymal cells for 14 days), ATP (10^-4 M) transiently increased the steady-state [Ca$_{++}$] levels from ~100 nM to 10 M without changing the internal pH. In parallel, ATP transiently uncoupled these cells as evidenced by the increase in the efficiency of the dye spread and by a reduction in the electrophysiological coupling between naive neuroblasts. These responses were observed during a neuronal developmental stage in which cholinergic and non-cholinergic neurons (in terms of ChAT expression for the former and side out patches and Ca$_{++}$ imaging). In contrast to the latter neurotransmitters, the magnitude of ATP-mediated responses progressively decreased as these cells underwent morphological maturation and were dramatically lower in primary neurons. Since uncoupling has been correlated with cellular differentiation and ATP-mediated events seem to be developmentally regulated, we are currently investigating whether ATP elicited responses are implicated in the expression of memetic excitability during ontogeny by neuronal activity.


Embryonic neuroblasts transplanted into regions of targeted photolytic neural cell death in neonates of juvenile or adult mice selectively migrate into the neuron-deficient regions up to 800 μm over a period of 1.2 weeks (B. Hennequin et al. 1992). We studied this migration directly in living slice preparations and investigate the potential role of radial glial cells as a structural substrate via in-emersion from postdevelopmental astrocytes and their axons. Nanoscale conjugated fluorescent dyes were injected into somatosensory cortex of 2-week-old mice. Two weeks later, centrolateral homotopic cortex received transcranial 640 nm laser illumination resulting in selective photolytic killing of photolabile antibody conjugates (Collazo et al. Science 264:426, 1994). Two days after transplantation fresh brains were cut at 150 μm, and the region containing the transplant was microdissected and processed for fluorescence. RC2 and GFAP immunocytochemistry 3, 7 and 14 days after transplant. Glial cells outside the injection site reacted positively for RC2 or GFAP at all ages studied, and a small number reacted for both. RC2 reactive cells resembled the GFAP reactive astrocyte, but had fewer processes and were fewer overall. GFAP reactive astrocytes in controls receiving transplants only or sham injection of H2O2 were found at all ages studied, but RC2 positive glia were present only within the injection site and only at 3 and 7 days and not at 14 days. These results suggest a role for RC2 positive glia during the selective migration of transplanted embryonic cells into neuron-deficient regions of young adult cortex. Further experiments are needed to determine if neurons associate closely with each other and whether these glia are of host or donor origin. Supported by HD29478, The Alzheimer’s Assoc., MR Center Grant HD16855, The Rita Allen Foundation, and an NSF Postdoctoral Fellowship to CHG.

NEURAL CREST AND PLACODES IN XENOPUS LAEVIS. A. Collazo*, J. Rohebo, M. Bromer-Franz, P. M. Maltes and S.E. Fraser. Beckman Institute 139-74, California Institute of Technology, Pasadena, CA 91125.

Our previous work has shown that neurons, the mechanoreceptors of the lateral line system of Xenopus laevis (Collazo and Berta) and Xenopus, are derived from both neural crest and epidermal placodes (Collazo et al. Science 264:426, 1994). We expand our analyses for Xenopus by using D13 (5') or 5'C18) to label small populations of neural crest, head mesoderm, placodal cells, and lysinum rhodamine dextran (LRD) to label individual neural crest cells. We used in vivo time lapse imaging of D13 labeled cells to study the migration of neural crest and placodes. Neural crest forming the first two branchial arches (mandible and hyoid) and last three arches originates, respectively, from midbrain and hindbrain. Crest fills the arches from ventral to dorsal and from crest cells contributing to the dorsal branch of the arch migrates later. We observed labeled neural crest cells in cartilage, neural, glial, connective tissue and neuramitans cells. The first neural crest cells to migrate tend to form mostly cartilage not neural structures. The neural crest cells that form the tube did not form neural crest; instead, labeled cells migrated within the neural tube to positions in the hindbrain. These data suggest an increasing differences of cell phenotypes from previous studies of neural crest in Xenopus (Sadaghiani and Thibault Dev. Biol. 124:91, 1987). We are unable to observe migration of neural crest before stage 18 and, at stage 15-16, the lateral population of "neural crest" is mostly head mesoderm. Development of the posterior and occipital lateral lines begins after stage 34. We observed labeled cells moving from one primordium (after the placode breaks up) to another along the lateral line.
D2 CYCLIN GENE FUNCTION IS IMPLICATED IN DIFFERENTIATION OF SPECIFIC NEURAL PRECURSORS IN BRAIN, LIP Identiﬁcation and P. Davis, Dept. of Neuroscience, University of Minnesota, Minneapolis, MN 55455.

Cyclins are regulatory proteins which promote the progression of dividing cells through the cell cycle. D2 cyclin expression is restricted to the transition from G1 to S phase and is thought to be widely expressed in mitotically active tissues. To investigate the potential role of D2 cyclin in neural precursor expression, we have isolated a neural cell line which exhibits this transition. D2 cyclin expression was not detected in the same neural cell line. These results suggest that D2 cyclin may play a role in neural precursor differentiation.

DEGENERATIVE DISEASE: ALZHEIMER'S—MECHANISMS OF CELLULAR INJURY II

TEA and Ca2+-SENSITIVITY OF K+ CHANNELS IN GEARFORMATOBLASTS FROM ALZHEIMER’S AND NORMAL DONORS. M. Baytak, R. L’Estrange, B. Whitehead, B. Reisinger, 1B, D. L. Altick, LAF, and T. Winblad, National Board of Health, Bethesda, MD 20892.

Our laboratory has identiﬁed K+ channel regulation as one of the critical steps in neural cell death. Because memory loss is the principal and earliest sign of Alzheimer’s disease (AD), and because AD may be systemic, we studied K+ channels in peripheral neurons and brain ﬁbroblasts. Using the cDNA complementary method, we have found that a 113 bp, intracellularly-sensitive (TEA)-sensitive channel is functionally active in ﬁbroblasts from AD patients. The present report extends our previous ﬁndings to human endothelial cells (HEC), which may be directly linked to the central nervous system (CNS) and express neural markers. Patch-clamp techniques were used to study K+ channels in HEC from AD patients and normal controls. In the cell activation mode, 57% of the control patches (n=30) and 56% of patches from AD cells (n=31) exhibited channel activity. The mean current density of the three K+ channel types (K+ 5), K+ 115, K+ 97, and K+ 197) was 21 ± 3.2 pA/pF. Each channel type was found with about the same frequency in active patches from AD and control individuals. These observations suggest that there may be a mechanism that is not only present in AD, but that also contributes to memory loss. The role of this mechanism in AD remains to be elucidated.

SSCP ANALYSIS OF TRANSFORMING GROWTH FACTOR Beta 3 IN THE CHROMOSOME 14 LINKED FAD PEDIGREES

S.M. Gaston1, W.H. Pettingell2, B. Pierre-Louis2, R.G. Feldman1, P. St. George-Hyslop1, R.E. Tanzi2

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Chromosome 14-linked Familial Alzheimer’s Disease represents a genetically well deﬁned form of this disease, characterized by Young-onset familial disease. However, only a small percentage of affected families have been linked to chromosome 14. Recently, we have characterized a large affected kindred that is not linked to chromosome 14. This kindred is characterized by mild symptoms and a longer disease course than typically observed in AD. Our study included the analysis of the tau gene, which is highly expressed in the brain and is thought to be involved in the pathogenesis of AD. We found that the tau gene is not expressed in the affected members of this kindred. These results suggest that the tau gene may not be involved in the pathogenesis of AD in this kindred. However, further studies are needed to determine the role of the tau gene and other genes in the development of AD.

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683.3 DISTRIBUTION OF APOLIPOPROTEIN E PHENOTYPES IN ALZHEIMER'S DISEASE. Dale M. VanderPutten1,2, Ginger S. Johnson2, Carl R. Merrill1. 1Laboratory of Biochemical Genetics, NIH, Washington, DC 20032, 2Monoclonetics Inc. Int., Houston, TX 77027, 3Molecular Geriatrics Inc., Lake Bluff, IL 60044

We have confirmed a risk of developing Alzheimer's disease (AD) associated with the apolipoprotein E (apoE) allele by phenotypic analysis of apoE4 protein in plasma, cerebrospinal fluid (CSF) and brain (z = 4.6). We have found significantly (p < 0.001) lower levels of apoE in AD patients' CSF but not in plasma, frontal cortex or hippocampus. ApoE4 levels in apoE4 homozygotic AD CSF were different from normal control levels. Analysis of apoE 3/4 heterozygotic individuals showed differences between apoE3 and 4 levels in AD CSF but not AD plasma or normal CSF. Quantitative RT-PCR analysis suggested the variation in relative amounts of apoE3 and 4 CSF results from differential metabolism apoE3 and 4 proteins in the AD CNS. We hypothesize this difference in apoE metabolism may be an important factor in development of AD.

683.4 RELATIONSHIP OF PROTEASE-RESISTANT PROTEIN, AMYLOID AND TUBULOFILAMENTOUS PARTICLES TO THE AGENT OF SPONSIFORM ENCEPHALOPATHY. H. Narvaez, Dept Psy, SUNY Stony Brook NY 11794.

Sporadic plaques, hallmark of Alzheimer's disease (AD), are also seen in Down's syndrome, and some transmissible spongiform encephalopathies (TSEs). The plaques and PPr are coded by two different normal genes. Unique tubulofilamentous particles (TP) seen in all TSEs have the triple layer structure: a paired helical filament (PHF) plaques observed in TSEs. The APP and PPr are coded by two different normal genes. Unique tubulofilamentous particles (TP) seen in all TSEs have the triple layer structure: a paired helical filament (PHF) plaques observed in TSEs. The APP and PPr are coded by two different normal genes. Unique tubulofilamentous particles (TP) seen in all TSEs have the triple layer structure: a paired helical filament (PHF) plaques observed in TSEs.

Apolipoprotein E (ApoE) allele E4 has been associated with late onset familial Alzheimer's disease (AD). In AD brain, ApoE is localized to the cerebrovascular lesions, amyloid plaque and neurofibrillary tangles. In a previous study we demonstrated that δ-amylloid protein precursor and α-antichymotrypsin, found accumulated in senile plaques in AD brain, were localized at neureumal junctions (NMJ). In the current study we asked whether ApoE was also localized at NMJ and was affected by denervation, using immunocytochemistry and Western blotting. The results indicate that ApoE was present in normal muscle at the NMJ, within intramuscular nerves, and on endothelial cell surfaces. Following axotomy, ApoE disappeared rapidly from intramuscular nerves. However, at the NMJ, ApoE lingered several days before disappearing. Such experiments in muscle after denervation may shed light on the mechanism of synaptic loss as well as plaque deposition in AD.

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683.12 OXIDATIVE DAMAGE TO MITOCHONDRIAL DNA IS INCREASED IN ALZHEIMER'S DISEASE. M.F. Beal*, U. MacGlavey, and P. Macovec. Neurology Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Oxidative damage to DNA may play a role in both normal aging and in neurodegenerative diseases. The mitochondria are the major source of free radicals in the cell, and recent evidence has shown decreases in cytochrome oxidase activity in Alzheimer's disease (AD), both in platelets and in cerebral cortex. Cytochrome oxidase inhibition leads to increases in free radical generation in isolated mitochondria. Our studies showed evidence for increased lipid peroxidation in AD postmortem brain tissues. We examined whether AD is associated with increased oxidative damage to both nuclear DNA (mDNA) and mitochondrial DNA (mtDNA) in postmortem brain tissue. We measured the oxidized nucleoside, 8-hydroxy-2-deoxyguanosine (OHdG), in DNA isolated from 3 regions of cerebral cortex and cerebellum in 13 AD and 13 age, matched controls. There was a significant 3-fold increase in the amount of OHdG in mDNA in parietal cortex of AD patients as compared with controls. In the entire group of samples, there was a small significant increase in oxidative damage to mtDNA and a highly significant 3-fold increase in oxidative damage to mtDNA in AD as compared with age-matched controls. These data suggest that mitochondrial DNA is particularly sensitive to oxidative damage, and that they show that there is increased oxidative damage to DNA in AD, which may contribute to the neurodegenerative process.

DUGS OF ABUSE: ADDICTION/TOLERANCE

684.1 THE RELATIONSHIP BETWEEN OPIOID AGONIST INTRINSIC EFFICACY AND TOLERANCE. Alokesh Duttaroy, Ka Wu Chan, Sokrut Shah and Byron C. Ying**. College of Pharmacy, St. John's University, Queens, NY 11439.

Tolerance is a relative consequence of repeated exposure to opioid agonists. Furthermore, the magnitude of tolerance has been shown to be dependent upon agonist intrinsic efficacy. Continuous infusions of high intrinsic efficacy compounds (e.g., fentanyl) produce less tolerance than infusions of lower intrinsic efficacy drugs (e.g., morphine). However, it has been reported that when opioid agonists are administered intermittently, the degree of tolerance is not significantly affected by intrinsic efficacy (Duttaroy et al., FASEB J., 7: A704, 1993). In the present study the role of intrinsic efficacy was investigated by comparing intermittent and continuous administration during protocols using 3 opioid agonists (morphine, etorphine and fentanyl). In the continuous administration study, mice were infused s.c. (osmotic minipumps) with 5 - 40 times the ED50 of each drug for 168hrs; the pumps were removed and 24hrs later mice were tested for analgesia (tailflick) in cumulative dose-response studies. Controls were implanted s.c. with inert placebo pellets. With increasing infusion doses, the magnitude of tolerance increased for all three agonists. However, maximal tolerance of ~3 fold was observed for fentanyl and etorphine at 40 times the ED50, whereas a 4 fold tolerance was produced by an infusion 10 times the ED50 for morphine. In another study, the effect of intermittent administration of morphine and etorphine on analgesic tolerance was examined. Mice were injected s.c. once per day for 7 days with saline or 10 times the ED50 for morphine or etorphine. 24hrs following the last injection mice were tested for analgesia. Although both drugs decreased the degree of tolerance was similar for both drugs. Intermittent morphine produced a 1.7-fold shift in the ED50, while intermittent etorphine produced a 2.1-fold shift in the ED50. These results indicate that under conditions of continuous administration intrinsic efficacy is a determinant of the magnitude of tolerance to opioid agonists. However, when studied intermittently, intrinsic efficacy appears to have little impact on tolerance. (Supported by NIDA DA 04183)


7-nitroindazole (NOS) attenuated some signs of opioid withdrawal (Kimes et al., Psychopharmacology 152:51-2, 1993). The purpose of the present study was to compare the potency of a brain-specific NOS inhibitor, 7-nitroindazole (7-NI) with that of less selective NOS inhibitors, L-N(omega)-nitroarginine (L-NNA), L-N(omega)-nitroarginine methyl ester (LNAME), and N(omega)-(1-iminoethyl)-L-ornithine (L-NIO). We also compared the attenuation of the withdrawal by NOS inhibitors to that produced by clonidine. Morphine dependence was produced by the subcutaneous implantation of 1 morphine pellet (75 mg) on Day 1. On Day 4, the following drugs (doses in mg/kg) were administered 1 h before saline challenge (0.5 mg/kg): L-NNAME (1, 3, 10, 30, and 100); 7-NI (1, 3, 10, 30, 60); L-NIO (1, 5, 10, 30, 60); clonidine (0.01, 0.03, and 0.1). All of the NOS inhibitors decrease wet dog shakes, weight loss, diarrhea, and grooming. The selective NOS inhibitor, 7-NI also attenuates the expression of other signs (maturation, salivation, and penis licks/jaculations), the expression of which are either unchanged or increased by the less selective NOS inhibitors. The expression of many signs are affected similarly by clonidine and 7-NI (weight loss, diarrhea, wet dog shakes, grooming and mastication), but clonidine uniquely attenuates the expression of abnormal posturing. The potencies of the NOS inhibitors to reduce the frequency of wet dog shakes during opioid withdrawal were similar for 7-NI and L-NNAME. L-NNA was almost 4-fold more potent, and L-NIO was less than half as potent as either 7-NI and L-NNAME. In conclusion, our results show that NOS inhibitors attenuate withdrawal at least as effectively, but not as potently, as clonidine, and suggest that 7-NI, which does not increase blood pressure, may be a good candidate for human use.
684.3 NALOXONAZINE PRETREATMENT IMPAIRS MORPHINE-INDUCED ACQUISITION OF PLACE-PREFERENCE BUT NOT STIMULATION OF DOPAMINE RELEASE Tanda G., R. Longoni, L. Spina, E. Carboni* and G. Di Chiara. Dept. of Toxicology Univ. of Cagliari (Italy).

Although opiates stimulate dopamine (DA) release and the firing activity of DA units, the role of DA in opiate reinforcement is debated. We now report that naloxonazine (15 mg/Kg i.p. 20 h before) fails to modify the stimulation of DA release in the arcuate nucleus elicited by morphine (1.0 and 5.0 mg/Kg i.c.) but prevents the acquisition of place-preference induced by a single pairing with morphine. The results suggest that DA does not mediate the primary reinforcing properties of opiates. However, since naloxonazine fails to extinguish heroin self-administration (Negus et al. JPET, 265, 1249, 1993) DA might act as a secondary reinforcer to maintain opiate self-administration.


We employed a three-chambered place preference test apparatus to examine the reinforcing and addictive potential of anabolic androgenic steroids (AAS) in adult Long-Evans male rats. Testing consisted of three phases. First, all rats received a pretest which allowed each animal 20 min to explore all three chambers and establish a place preference. For the second (conditioning) phase, the animals were divided into two groups: an experimental group receiving twelve daily injections of 1mg testosterone propionate (TP) alternating with propylene glycol (PG), and a control group receiving only PG. Injections were given 15 min prior to placement in a chamber associated with either TP or PG for 45 min. In the third phase the rats received two place preference tests. In the first test all rats received PG and time spent in each chamber was recorded. In the second test they were given either TP or PG and tested again for place preference. Results showed that rats receiving TP paired with a particular chamber spent significantly more time in that chamber. PG-treated rats did not show a place preference. The potential for AAS addiction is suggested by the findings that 1) rats can discriminate the presence of AAS from a control substance, and 2) that AAS are positively reinforcing in a place preference test. (supported by a grant from the Harry Frank Guggenheim Foundation)

684.5 EVIDENCE THAT IBOGAINE INHIBITS DOPAMINE RELEASE VIA A KAPPA RECEPTOR MECHANISM. Malcolm S. Reid*, Kang Hu, Patricia Beadorick, S. Paul Berger, UCSF/VAMC, Substance Abuse Treatment Research 116W, 4150 Clement St. San Francisco, CA 94121; Pharmacology Dept, CUNY Medical School, 138th St and Convent Ave, New York, NY 10031.

Ibogaine has been proposed as a clinical treatment for stimulant and opiate addiction. In the present study in vitro microdialysis was used to determine the effects of ibogaine on the extracellular levels of dopamine in the nucleus accumbens and striatum of freely moving Sprague-Dawley rats. In addition, the effects of the non-selective opiate antagonist naloxone and the kappa receptor agonist opioid antagonist norbinaltorphimine (NorBNI), alone and in combination with ibogaine, were also studied. All drugs were tested locally by perfusing them through the microdialysis probe. Ibogaine was tested at several doses (10-6M to 10-3M) and it was found that it produced a biphasic dose-response curve, whereby lower doses (10-6M to 10-4M) produced a dose-dependent decrease in dopamine levels while higher doses (5x10-4M and 10-3M) produced an increase in dopamine levels, seen in both the nucleus accumbens and striatum. Naloxone and NorBNI (10-6M to 10-4M) had no effects on dopamine, except the highest dose of naloxone (10-4M) which produced an increase in dopamine levels. Co-administration of naloxone or NorBNI (10-6M or 10-4M) with ibogaine (10-4M) blocked the decrease in dopamine levels produced by ibogaine alone, suggesting that the activity of ibogaine to inhibit dopamine release is mediated by kappa opiate receptors. Further studies on the dopamine stimulatory properties of ibogaine are underway.

684.6 \(\Delta^9\)-TETRAHYDROCANNABINOL AND THE SYNTHETIC CANNABINOID CP-55,940 INDUCE EXPRESSION OF c-fos mRNA IN STRESS-RESPONSIVE NUCLEI OF RAT BRAIN. M. Herkenham* and L. S. Brady. Section on Functional Neuroanatomy, Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892.

Little is known about the functional consequences of acute cannabinoids (marijuana) intoxication. Rats were injected acutely i.p. with \(\Delta^9\)-tetrahydrocannabinol (10 mg/kg) or the synthetic cannabinoid CP-55,940 (1, 3, or 10 mg/kg) and sacrificed 40 min later. Brains were processed for in situ hybridization of the riboprobe for the immediate-early gene c-fos. Induction of c-fos mRNA expression occurred in a set of structures that comprise the central effectors of the stress response—paraventricular nucleus of the hypothalamus, anterior lobe of the pituitary, locus coeruleus, ventral lateral septum, central nucleus of the amygdala, and paraventricular nucleus of the thalamus. The two drugs produced similar patterns of responses, and responses to CP-55,940 were somewhat dose-dependent. Motor structures, notably those containing dense cannabinoid receptors, did not show elevated c-fos mRNA levels. Thus the activated structures reflect the animals' psychological (cannabinoids elevate plasma corticosterone and appear to be stressors in rats) and motoric states (cateleptic and not the primary sites of action of the drug.

684.7 DELETION OF \(\beta\)-ENDORPHIN IN THE MOUSE BY TARGETED GENE MUTAGENESIS. M. Rubinstein, M. Iapò, E. C. Chan, R. Allen* and M. J. Law. The Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201, U.S.A.

The endogenous opioid peptides are proposed to modulate neuroendocrine circuits, autonomic reflexes, analgesia, memory, and learning through binding to a family of G-protein coupled receptors. Our laboratory has initiated a program to test the function of the individual components of the endogenous opioid system by gene targeting in embryonic stem cells. \(\beta\)-endorphin is produced in three discrete areas: the arcuate nucleus, the nucleus tractus solitarius, and the pituitary by the posttranslational processing of proopiomelanocortin (POMC). Because POMC is also important as a precursor for ACTH and melanocyte stimulating hormones (MSH), we designed a gene targeting vector that encodes a truncated POMC prohormone selectively deficient in the \(\beta\)-endorphin peptide sequence (Nucleic Acids Research 21:2613-2617, 1993). Mice homozygous for the mutated POMC allele are developmentally normal, grow to maturity and reproduce. HPLC chromatography and RIA of hypothalamic and pituitary extracts show that the truncated POMC prohormone is authentically processed to ACTH and MSH in the total absence of \(\beta\)-endorphin-like immunoreactivity. As a result, the mice have normal hypothalamic-pituitary-adrenal function under basal and restraint-stress induced conditions. The neurons that normally produce \(\beta\)-endorphin are intact and the wide-spread fiber distribution from these neurons is also normal and detectable with an antisem to ACTH on brain sections. Preliminary data suggest that swim-stress induced analgesia is unaltered in the \(\beta\)-endorphin deficient mice. Additional behavioral tests are ongoing. We conclude from these studies that \(\beta\)-endorphin is not an essential neuromodulator for development of the nervous system or organization of the neuroendocrine systems that control the stress reponse.
65.1 EXCITATORY-NEURONAL TROUBLE IN THALAMUS PREDICTS MOTOR SEIZURES IN A RAT MODEL OF WERNICKE'S ENCEPHALOPATHY. P.J. Langhans*, S.X. Zhang, G.S. Weisskoff, Department of Psychiatry, and Neuroradiology, Department of Neurological Surgery, School of Medicine and Dentistry of the University of Rochester, Rochester, NY 14642.

We have previously shown that rats that died of Wernicke's encephalopathy (WE) had decreased NMDA receptor protein levels in the thalamus. The purpose of the present study was to determine if Wernicke's encephalopathy is predictive of motor seizures in a rat model of Wernicke's encephalopathy. Thalamic NMDA receptor protein levels were measured in rats treated with 3-NP (approximately 3 mm in diameter and 26 mm in volume) by Western blotting. We found that rats that died of WE had decreased NMDA receptor protein levels in the thalamus. These findings are consistent with the hypothesis that brain damage in human alcoholics may be mediated by an unleashing of the excitotoxic potential of endogenous Glu. Supported by AA 07466, AO 05681, MH 14677, NS 29481, and RSA MH 38984.

65.2 HYPERACTIVATION OF GLUTAMATE PATHWAYS INDUCES THALAMIC CYTOTOXOPATHY RESEMBLING WERNICKE-KORSKOFF ENCEPHALOPATHY. J.W. Olney*, J.A. Corso, A. Labouret, S.X. Zhang, P.J. Langlais, Washington Unv., St. Louis, MO 63110 and San Diego State University, San Diego, CA 92182.

Wernicke-Korsakoff encephalopathy (WKE) is a brain damage syndrome associated with alcoholism and thiamine deficiency (TD). Feeding a thiamine deficient diet to rats pretreated with 3-NP induces a TD brain damage syndrome that closely resembles both the pattern and type of cytopathology seen in WKE. Acute neuron-necrotizing lesions in various thalamic nuclei is a unique and consistent feature of WKE. We studied the effects of 3-NP treatment on the thalamic TD syndrome in rats. Langlais et al (1990,1993) have shown in the TD rat that there is a striking elevation of extracellular glutamate (Glu) in vulnerable thalamic regions as the lesional process is developing, and that these increases can be prevented by pretreatment with MK-801, a powerful antagonist of NMDA Glu receptors. In the present study, we have performed an electromicroscopic analysis of the acute cytopathology of 3-NP treated rats and compared it with acute cytopathology induced in VPL by hyperactivation of Glu-ergic thalamocortical afferents to VPL. In both of these syndromes the cytopathological reaction was found to have the characteristic features of Glu-induced excitotoxicity. Acute edematous swelling of pyramidal dendrites and neuronal cell bodies, degenerative changes in mitochondria and endoplasmic reticulum and swelling of nuclear chromatin. In both syndromes, as does sparing of pyramidal axon terminals. These findings are consistent with the hypothesis that brain damage in human alcoholics may be mediated by an unleashing of the excitotoxic potential of endogenous Glu. Supported by AA 07466, AO 05681, MH 14677, NS 29481 and RSA MH 38984.


Several neurodegenerative diseases are characterized by selective neuronal losses which may result from a defect in energy metabolism. We hypothesize that a selective impairment of mitochondrial function resulting in decreased ATP production, increased glutamate release, and resultant neuronal death may be a feature of various neurological diseases. We have previously shown that the mitochondrial membrane potential (DeltaPsi) is decreased in cells obtained from the skin of patients with diseases characterized by selective neuronal death. We also have shown that mitochondrial membrane potential is decreased in cells obtained from the skin of patients with diseases characterized by selective neuronal death.
685.8 GLUTAMATE RECEPTOR MEDIATED DOPAMINERGIC TOXICITY DUE TO ER INHIBITION. O.O. Beverskold, L. Declercq-Felin and Y.O. Stillman. Neurology, U01NS3-062, UCSD, San Diego, CA 92103.

Metabolic deficiencies have been detected in patients with Huntington's, Parkinson's and Alzheimer's diseases suggesting a metabolic defect as a common etiology. Features of Huntington's disease, including administration of an irreversible succinate dehydrogenase (SDH) inhibitor, with or without a reversible SDH inhibitor, malate, produced lesions that were "excitotoxic". Based on these findings, abnormal metabolism could trigger an excitotoxicity. (J. Neurochem. 61:11147 - 1115, 1993, 1993). In this study, we examined the possible involvement of glutamate receptor-mediated excitotoxicity in Huntington's disease. The 

685.9 GLUTAMATE-ELICITED (Na+*) INCREASE DAMAGES NEURONAL CALCIUM BUFFERING BY MITOCHONDRIA. L. Kiedrowski* and E. Costa-Faria-Georgetown Institute for the Neurosciences, Georgetown University, Washington, DC.

Exposure of primary cultures of cerebellar granule cells to glutamate (50 μM) applied in a Mg2+ -free medium for 4 min increases somatic Na+*, up to 200 nM and after glutamate removal (Na+*), fails to decay promptly. In contrast, Ca2+* decreases by 5 μM decreases to 1.5 μM decreases immediately after glutamate exposure. Moreover, a sudden increase of extracellular Ca2+* follows immediately after glutamate removal when Na+* is still elevated. These data indicate that mechanisms other than the plasma membrane N2 Ca2+* uptake and extracellular Ca2+* stores and other mechanisms in somatic Na+* that affects the Ca2+* buffering efficiency. In this study, we examined the effect of glutamate on Na+*, Ca2+*, and Cl- buffering efficiency. In this study, we examined the effect of glutamate on Na+*, Ca2+*, and Cl- buffering efficiency. In this study, we examined the effect of glutamate on Na+*, Ca2+*, and Cl- buffering efficiency.

Recent studies have suggested that bombesin acts to inhibit sodium appetite caused by depletion of sodium (Soc. Neurosci. Abstr. 19: 1263, 1993; Soc. Neurosci. Abstr. 19: 1822, 1993). Rats with lesions centered on the AP (AP-lesions) are reported to consume increased amounts of sodium (Nat. J. Physiol. 264: R1242, 1993). Since the AP is a circumventricular organ and has a blood-brain barrier it is possible that bombesin acts in the AP to inhibit sodium appetite. When treated with 4 μg/kg bombesin I.P., rats with AP-lesions decreased their 2h need-free intake of 2% saline compared to 2% saline intake when injected with sterile physiological saline I.P. (P < 0.02). In addition, bombesin (4 μg/kg I.P.) reduced sodium depletion-induced 2% saline intake in both AP-lesioned and unlesioned rats (P < 0.05). Conversely, water intake induced by isoproterenol (25 μg/kg) was not reduced by bombesin in either rats with lesions of the AP or intact control rats (P > 0.05). These data indicate that bombesin is effective at reducing sodium appetite in rats with AP-lesions. Thus, sites other than the AP are capable of mediating the effects of peripherally administered bombesin on sodium appetite. (Supported by NIH DK 42533)

686.5 UNCONDITIONED AND CONDITIONED EXPRESSION OF cFOS TO AMPHETAMINE AND LITHIUM CHLORIDE IN RAT BRAIN DURING TASTE AVERSION LEARNING. M.W. Swank, G.E. Smith and L.L. Bernstein. Department of Psychology, University of Washington, Seattle, WA 98195.

Amphetamine and lithium chloride (LiCl) are both effective unconditioned stimuli (USs) in the formation of learned taste aversions. However, the mechanism of action of these drugs is quite different with the area postrema and related emetic circuitry critical to the response to LiCl but not amphetamine. Induction of Fos-like immunoreactivity (FLI) in rat brain was examined two hours following ip injection of either d-amphetamine sulfate (3 mg/kg) or LiCl (6 ml/kg). Amphetamine strongly induced FLI in striatum while LiCl did not. In most other regions where FLI was detected (e.g. nucleus tractus solitarius (NTS); lateral septum; paraventricular nuclei of the thalamus and hypothalamus) substantially more FLI was noted in animals treated with LiCl than in those treated with amphetamine. In the lateral pontine parabrachial nucleus (PBN), however, d-amphetamine and LiCl induced comparable, significant elevations of FLI. After a single prior pairing with either LiCl or amphetamine, exposure to a saccharin solution induced significant taste aversions and significant FLI in intermediate NTS relative to unpaired controls. Overall, these results indicate that PBN is a site of common activation by these two very different drugs during acquisition. The response to taste re-exposure points to a population of cells in intermediate NTS which have a common role in taste aversion expression despite different US activation patterns.


The neural basis for the feeding-related increase in corticosterone (CORT) was explored using the chronic decerebrate (CD rat) in which the caudal portion of the brainstem is neuraly isolated from the forebrain by a complete transection of the neuraxis. Tail blood samples were taken before and after a gastric intubation of either 8 ml milk/diet or water following overnight deprivation. Pre-intubation baseline CORT levels were comparable in CD and intact controls. In intact rats, CORT increased significantly with the water intubation (110% above baseline). By contrast, no water response was observed in the CD rat. Both groups, however, showed a dramatic elevation in CORT following the nutritive load (intacts: 350% above baseline; CD: 440% above baseline). Additionally, tail blood samples were taken 1 hr after each of 5 intubations of milk diet spread evenly throughout the 12 hr light cycle. Cd rats showed CORT levels that were slightly elevated from those of the intact controls, but the rise and fall of CORT levels through the light cycle in CD rats exactly paralleled those of the intact controls. The CD rat’s normal-like CORT profile may reflect the competence of the HPA axis in the transition to a state of acute stress, and the function of the HPA axis can be modulated by humoral signals associated with food deprivation and caloric loads. Alternatively, the CD rat's normal-like CORT profile may have been mediated by the autonomic nervous system without a significant forebrain contribution.

686.7 DISTRIBUTION OF FOS-LIKE IMMUNOREACTIVITY IN RAT BRAIN FOLLOWING ISOPROTERENOL TREATMENT AND THE EFFECTS OF CONVERTING ENZYME INHIBITION OR ANGIOTENSIN II BLOCKADE. M. McGlynn, B.J. Outfield. Howard Florey Institute of Experimental Physiology and Medicine, Univ. of Melbourne, Parkville, 3052, Vic., Australia.

Subcutaneous (s.c.) administration of the β-adrenergic agonist isoproterenol causes copious water drinking in rats which is blocked by angiotensin II inhibition. The aim of the present experiment was to map the distribution of neurons in the brain of the rat which increased Fos production in response to isoproterenol and to test the effect of the angiotensin II antagonist losartan or the converting enzyme inhibitor captopril (at 2 different doses) on this response. Rats were injected with isoproterenol (Winthrop, 50μg/kg s.c.) and killed 2h later. Immunohistochemistry was used to detect Fos-like immunoreactivity (Fos-LI) in parformaldehyde-fixed sections of brain. Fos-LI was increased in neurons in the lateral terminals, in particular those in the sublominal o颍an (SFO), median preoptic nucleus (MnPO), and organum vasculosum of the laminae terminals (OVLT). It increased also in the hypothalamic supraoptic and paraventricular nuclei, bed nucleus of the stria terminals, central nucleus of the amygdala, locus ceruleus, parabrachial nucleus, nucleus tractus solitarii, ventrolateral medulla and area postrema, but not in control rats injected with s.c. isotonic saline. Locartan (Du Pont-Merck, 100mg/kg, intraperitoneal) or captopril (Bristol-Myers Squibb, 50mg/kg s.c.) which block isoproterenol induced drinking, also blocked Fos-production in the lateral terminals, but not in the other regions.


The anorectic responses to imbalanced amino acid diets (IMDI) have been associated with neural activity in the prelippom cortex (PPC), an area critical for the expression feeding responses to IM. Because the principal neurons of the PPC contain glutamate, we measured the intake of IMD after acute injection of the NMDA receptor antagonist, D.S -(1)-2-Amino-5-phosphono-phorbonic acid (AP5), into the PPC. After stereotaxic placement of bicuculline aimed at the PPC and prefeeding a low-protein basal diet (BAS) for 10 days, rats were given 0.5 μl of either AP5 (4 mM in artificial CSF, N = 11) or vehicle (CSF, N = 8) just before feeding IM at the onset of darkness. BAS intake on the day prior to injections was taken as control for each rat. Coordinates for the PPC were: A 11.4, L 3.7, D 6.5 (Paxinos and Watson, 1982). Intake of IMD was increased to 83% of control in the AP5 group (p = 0.024 vs CSF group, that ate 55% of control) at 6-9 hr after introduction of IM, a crucial time period in the feeding responses. Intake values for 6-9 hr were: AP5 group = 3.59 ± 0.41g, vs CSF group = 2.29 ± 0.38g, (p = 0.038). Although feeding at other time points was not affected, the 6-9 hr increase was sufficient to increase intake of IMD nearly to a significant level at 24 hr (p = 0.058). We conclude that the NMDA receptor in the PPC may have a role in mediating the anorectic responses to IMD diets. Supported by USDA CSRS 90-37200-5440, NIH DK35747 and DK42274.
686.9

STIMULATION OF FEEDING BEHAVIOR IN THE RAT BY INTRA-
HYPOTHALAMIC INJECTION OF 8-BR-CAMP. E.R. Gillard, A.M. Khan, A.U. Has, R.S. Gwirali, and B.G. Stanley. Departments of Neuroscience & Psychology, University of California, Riverside, CA 92521, USA

Although several neurotransmitters acting in and around the lateral hypothalamus (LH) have been implicated in the control of eating, it is unclear which, if any, second messengers mediate their effects. To begin to address this, food intake was measured 1, 2, and 4 hrs after injection of 8-
rhodanodine 3,5-cyclic monophosphate (8-BR-CAMP, a membrane-
permeant cAMP analogue) into the perifornical hypothalamus (PFH) of freely feeding adult male Sprague Dawley rats. Each dose of 8-BR-cAMP (0, 1, 10, 50 and 100 nmol, dissolved in artificial cerebrospinal fluid vehicle) was administered in a final injection volume of 0.3 μl. Dose-dependent stimulation of eating was observed, with doses of 50 nmol and 100 nmol eliciting average 2 hr intakes of 3.9 and 15.7 g, respectively. To determine the locus of this effect, we compared the effects of 8-BR-cAMP injected into the PFH and six other areas bracketing the PFH (LH, anterior and posterior LH, paraventricular nucleus of the hypothalamus, amygdala, and thalamus). Consistent eating-
stimulatory effects were observed in the LH and PFH at doses ≥ 50 nmol (2 hr intakes ≥ 3.4 g). Thalamic injections resulted in a more variable feeding response which achieved significance only at 100 nmol. In contrast, injections into all other sites failed to stimulate eating. These results suggest anatomical specificity of 8-BR-cAMP’s effects, with likely sites of action being the LH/PFH and/or thalamus. It is possible that neurotransmitters known to influence eating in the hypothalamus may do so via the cAMP second-messenger system.

686.10

HYPOTHALAMIC GALANIN IN RELATION TO CIRCULATING INSULIN AND FAT INGESTION. S.F. Leibowitz, J.J. Koening and A. Akabasahi. The Rockefeller University, N.Y., N.Y. 10021 and The Immunology Research Institute, Annapolis, N.J. 08081

Galanin (GAL) is densely concentrated in neurons of the hypothalamic paraventricular nucleus (PVN). When injected into the hypothalamus, GAL reduces insulin secretion and stimulates feeding behavior, causing a preferential increase in fat ingestion. The present studies in albino rats reveal an inverse relationship between endogenous GAL peptide (measured via RIA), specifically in the PVN, and circulating levels of insulin, while GAL and fat ingestion are positively related. Three experiments were conducted: 1) With measurements taken at several intervals across the light/dark cycle (8 time points, n=7/time), GAL levels in the PVN, as opposed to other hypothalamic nuclei, increased significantly during the middle phase of the feeding cycle, while insulin levels were declining and natural fat ingestion increased. PVN GAL and circulating insulin levels were inversely correlated (r=-0.43, p<0.05) across the feeding cycle. 2) In rats (n=28) on macromounted diets, GAL levels in the PVN, but not other hypothalamic areas, were inversely correlated with circulating insulin levels (r=-0.41, p<0.05) but positively related to daily fat intake (r=+0.64, p<0.01). 3) In rats (n=11) given 5 daily injections of GAL antisept oligonucleotides into the PVN, GAL levels and daily fat intake decreased by 35% (p<0.05 compared to sense controls), while circulating insulin levels tended to rise (+46%, p<0.10). This inverse relation between endogenous GAL and insulin under normal feeding conditions is consistent with another report at this meeting (Tang et al.) showing insulin in diabetic rats to reduce GAL gene expression and peptide levels, specifically in the area of the PVN.

686.11

THE RELATIONSHIP OF AMOUNT OF SOUP IN THE STOM-
ACH TO THE CONTROL OF TEST MEAL INTAKE IN MEN AND
WOMEN. R.S. Kussinoff, R. Wastoff, D. P. Pierson, and F. X. Pi-Benhy.
New York Obesity Research Center, St. Luke’s-Roosevelt Hospital and

If a critical level of gastric tension, induced by volume, triggers the cessation of eating, graded volumes of food stored should be the migrants in intake in a subsequent test meal, thereby preserving constancy of total (preload + meal) volume, as preload size increases. This hypothesis holds only if meals and preloads are at the same rates. To test this hypothesis, five women and six men in good health, nonobese and without medical problems, ate a yogourt shake test meal 30 min after tomato soup preloads. Emptying of the preload from the stomach was measured by radionuclide scintigraphy (see table for the volume of soup remaining at the start of the test meal). The preloads and test meals were given with at least two days between. Preload sizes ranged from 160 to 800 ml in 160 ml steps. No preload condition was included. Test meal intake was reduced as a function of preload size in a linear fashion only between 320 g and 640 g in women and only at the 160 g compared to 0 and 800 g compared to 640 g preload volumes in men (See Table below for details).

Preload (g) 0 160 240 480 640 800 1600
Women intake (g) 0 160 240 480 640 800 1600
Women preload (g) 0 23 65 194 265 265 110

Unless the preloads of soup differentially affect test meal eating, gastric tension, induced by volume, by itself, is not a critical signal for normal meal termination in humans. (Support: NIH - DK35007 & DK80867)

686.12


Migratory birds rely on increased fat storage and fatty acid utilization to meet seasonal changes of energy expenditure and as a result increase fat reserves. To determine if their feeding behavior is sensitive to carbohydrate utilization, white-crowned sparrows (Zonotrichia leucophrys gambelii) maintained on short daylength (9L15D) were injected with 2-deoxy-D-glucose (2-DG), 2,6-anhydro-D-mannitol (2,6-AM), or insulin, drugs that elicit feeding in mammals. Low doses of 2-DG (25 or 50 mg/kg) had no effect on food intake and higher doses (100 or 300 mg/kg) significantly suppressed feeding after 1 and 2 hours. No dose of 2-DG increased meal size. Analogously, low doses of 2,6-AM (25, 50 or 100 mg/kg) had no effect on food intake, and higher doses (300 and 600 mg/kg) significantly suppressed intake. Low doses of insulin (0.5 or 1.0 U/kg) also had no effect on food intake and higher doses (2.0 or 4.0 U/kg) significantly suppressed feeding and induced hypoglycemia after 1 and 2 hours. These data suggest that decreased carbohydrate metabolism does not elicit feeding in these species, and after injection of 2-DG and insulin, and that suppression of food intake was caused by the decrease in circulating glucose levels.

686.13

EATING SUPPRESSION AND BODY WEIGHT LOSS BY LATERAL
HYPOTHALAMIC (LH) NMDA RECEPTOR BLOCKADE WITH D-AP5. B.G. Stanley, V.L. Willett III, H.W. Donia and M.G. Dei II. Departments of Neuroscience & Psychology, University of California, Riverside, CA 92521.

We have recently shown that interest eating is elicited by LH injections of glutamate, N-methyl-D-aspartic acid (NMDA), or non-NMDA receptor agonists (Brain Res. 1993, 633, 88-95 & 638, 41-49). To examine whether LH glutamate and NMDA receptors might mediate natural eating, LH injection of the competitive NMDA receptor antagonist D-AP5 (10 nmol, 0.1 μl of artificial CSF) was tested for anorectic effects in adult male Sprague-Dawley rats. First, we examined whether pretreatment with LH injection of D-AP5 would suppress eating elicited by LH NMDA (10 nmol) and 15.7 g, respectively.To determine the effects of long-term treatment, D-AP5 was bilaterally injected into the LH at the onset of the dark-phase for 8 consecutive days. Compared to vehicle injection, D-AP5 produced marked and prolonged reductions of daily food intake and loss of body weight. Food intake normalized when the injections were stopped. These findings suggest that endogenous LH glutamate may act in part via NMDA receptors to influence eating behavior and body weight.
687.1
CMT ENHANCES SPROUTING IN PARTIALLY DENERVATED MUSCLE. S.G. Siegel*, A.W. English, Dept. of Anatomy & Cell Biology, Emory University, Atlanta, GA 30322.

Following partial denervation of skeletal muscles, the remaining intact motorneurons develop sprouts which innervate a limited portion of the denervated fibers. This response is thought to contribute to the regeneration of the lateral gastrocnemius (LG) muscle receives innervation from precisely one branch of the muscle nerve. After partial denervation by transection of the branch to the medial compartment (ULm) of the LG muscle, newly formed sprouts do not cross the compartmental border. To investigate whether exogenous neurotrophic factors (CNTF) can augment the natural sprouting response, rat LGm fibers were denervated. CMTF was administered by intramuscular injection (2-20ug/kg) into the LG muscle once daily for 1 week. Animals were sacrificed 1 day after the last injection, and the tissue was analyzed for sprouts. The motor end plates and nerve terminals were visualized immunohistochemically by the presence of clustered fluorescent alpha-bungarotoxin and FITC-conjugated antibody to the 200kd neurofilament protein, respectively. Sprouts from intact motorneurons were identified as thin offshoots from the nerve terminals or preterminal axon. CMTF administration resulted in an increase in the proportion of end plates with sprouts, suggesting that the natural sprouting response to partial denervation can be enhanced. However, sprouting from motorneurons in the intact compartment did not lead to reinnervation of fibers in the denervated compartment.

CMTF was provided by Regeneron Pharmaceutical, Inc.

687.2
ANTEROGRADE INFLUENCES ON COLLABORATOR NEURONAL SPROUTING IN THE SYMPATHETIC NERVOUS SYSTEM. G. A. Kuchel*, C. Richard and M.C. Bastian. Montreal General Hospital Res. Inst., 1655 Cedar Montreal, Quebec,Canada H3G 1A4

In contrast to retrograde signals for collateral growth (ie. target-derived NGF), anterograde influences on this form of plasticity are less well delineated. Using a NE-uptake assay, Dornay et al. (J. Soc. 5:1340, 1985) concluded that an intrinsic innervation was essential for the development of collateral sprouting in the rat pinal gland after a unilateral denervation. This target receives bilateral innervation from the two superior cervical ganglia (SCG). We have quantified fluorescent immunohistochemistry for tyrosine hydroxylase (TH) as an index of collateral sprouting (Exp. Neuro 124:381).

As shown earlier, 1 day after a unilateral denervation (R-ICN cut) the rat pinal gland exhibited a 50% decrease in the area fraction representing TH immunoreactive profiles (TH-IR). Collateral sprouting then took place with an increase to 78% and 76% of control values at 3 and 10 days, respectively. When this lesion was immediately preceded by a decentralization of the "intact" SCG (L-CSTx), TH-IR was 71% and 72% of control values at 3 and 10 days, suggesting that much of the collateral sprouting response was still intact. Further studies will be required to evaluate the possibility that degenerating preganglionic neurons "releasing" neurotransmitters during critical periods of signaling supplant usual anterograde signals for lesion-induced postganglionic collateral sprouting.

687.3

Lumbosacral dorsal rhizotomy, sparing one root, induces sprouting by that root in adults (Polistina et al., 90). Scatic nerve section enhances regeneration of the central process of crushed dorsal root in adults (Richardson et al., 87). Scatic nerve lesions are also accompanied by decreased synthesis of CGRP and SP by DRG neurons and depletion of these peptides from the dorsal horn (Nohias et al., 93; Himes and Tessler, 89). In neonates scatic lesion induces sprouting by central processes into the dorsal horn unaccompanied by depletion of peptides (Reynolds, R.J. et al., 90). We combined unilateral scatic nerve section with bilateral spared root preparation to determine whether the peripheral lesion would enhance sprouting by the spared root in adult rats. The immunocytochemical staining was compared on the two sides using antibodies against neuropeptidase and cytoskeletal proteins. CGRP and SP levels increased on the scatic nerve lesioned side at 3 days and the CGRP projection was expanded, SP returned to normal (or less) at 10 days and CGRP returned to normal (or less) at 20 days, indicating a transient effect of scatic lesion in this paradigm. The sprouting of central projections however blocks or delays the downregulation of peptide synthesis normally evoked by peripheral nerve lesion. Immunochemical staining of cytoskeletal proteins high molecular weight (BMW) tau, MAP 1B and phosphorylated MAP 1B showed no significant differences, suggesting that central sprouting in this paradigm is not associated with axonal elongation.

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687.4
SPROUTING OF ANTIFERRENT APPLETS IN THE DENERVATED CUNEATE NUCLEUS OF ADULT RATS AFTER DORSAL RHIZOTOMY. P.E. Garrapathy1,2, D.L. Sengelaub1, A.C. Mills1 and N. Mujad1.


In 1991, Pons et al. (Science, 252, 1857) reported a remarkably extensive topographic reorganization in SI (area 3b) of macaque monkeys that had undergone dorsal rhizotomies extending from the second cervical (C2) to the fifth thoracic (T5) level some years earlier. Here, we evaluate the hypothesis that this reorganization was due to the formation of new connections between intact afferents and nerves that had been degenerated by the rhizotomies. Thus, we have performed comparatively extensive, dorsal rhizotomies in adult rats followed by anatomical tracing and electrophysiological mapping experiments. Using peripheral skin injections of a conjugate of cholera toxin subunit B and horseradish peroxidase, we have observed aberrant expansions of cervical projections into the cuneate nucleus within 3 months of the deafferentation. Moreover, this new growth apparently forms functional connections because cortical recordings in forelimb regions of SI in these same animals reveal neurons that are responsive to stimulation of hindlimb skin surfaces innervated by afferents that normally project to the gracile nucleus. These results suggest that substantial new growth is possible in the mature central nervous system, and that such new growth may well account for the substantial functional recovery detected in earlier experiments in the so-called "Silver Springs monkeys." (Supported by S07 RR07031)

687.5
PROGRESSIVE ENTORHINAL LESIONS ACCELERATE HIPPOCAMPAST SPROUTING IN RATS. T.S. Carrigan, M.L. McGuilkin, and J.L. Ramirez.

Neuroscience Program and Department of Psychology, Davidson College, Davidson, NC 28036.

Entorhinal cortex (EC) lesions are known to induce sprouting by the crossed temporopontine pathway (CTP) in rats. Previous evidence from our laboratory indicates that two-stage (progressive) lesioning of the entorhinal area accelerates recovery from spatial alternation deficits associated with unilateral EC lesions and increase the synaptic efficacy of the CTP by 250% as little as 6 days postlesion.

The purpose of the study was to determine automatically whether progressive lesions accelerated CTP sprouting. Animals were assigned to one of four groups: progressive lesion, control, priming lesion, or one-stage lesion. The postlesion period in the CTP was analyzed using a two-way analysis. The CTP sprouting response was quantified by creating a ratio of the silver grain density evident in the outer molecular layer of the dentate gyrus contralateral and ipsilateral to the H-222 injection (Ci). The Aadium was to probe the best possible control lesion, the priming lesion to induce a significant sprouting response at 6 days postlesion. Progressive lesions increased the Ci ratio by approximately 300% in both the dorsal and ventral layers of the dentate gyrus. The lesion sizes of the two-stage and progressive treatment conditions were similar. We conclude that progressive entorhinal lesions enhance the rate of sprouting by the crossed temporopontine pathway. Taken together, the histological, electrophysiological, and behavioral results indicate that CTP sprouting is functionally significant and behaviorally meaningful.

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687.6

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Organotypic slice cultures of murine hippocampal tissue provide an in vitro model system for the controlled study of mossy collateral sprouting into the dentate molecular layer(DML). We hypothesized that the presence of afferent innervation would decrease the degree of mossy fiber collateral sprouting in the DML. Alternate slices derived from the mid-hippocampal region were cultured with or without attached entorhinal cortical tissues. Bodian silver staining confirmed the presence of mossy fibers in both the dentate gyrus and entorhinal cortex. Timm’s heavy metal staining and densitometry were utilized to assay the degree of mossy fiber sprouting. Attached entorhinal cortex significantly reduced the degree of mossy fiber sprouting in the DML after ten days in vitro (p<0.003; n=30 pairs). To confirm that damage to mossy fiber axons was not responsible for mossy fiber sprouting in the DML, alternate pairs of hippocampal slices were subjected to either complete transection of their mossy fiber projection (at the point where the fibers leave the hilus) or left undisturbed. Slices receiving mossy fiber lesions displayed no significant increase or decrease in mossy fiber sprouting into the DML as compared to adjoining control slices receiving no mossy fiber lesions (n=10 pairs). Therefore, mossy fiber collateral sprouting into the dentate molecular layer is not a consequence of damage to mossy fibers, but rather, may be regulated by the presence of afferents from the entorhinal cortex in vitro. Supported by DOE grant #2-89/116/38-150.
DELAYED DEGENERATION DIFFERENTIALLY AFFECTS THE TIME COURSE OF SPROUTING BY AFFERENT SYSTEMS REMAINING IN THE MOLLEcular LAYER OF THE DENTATE GYRUS FOLLOWING LESIONING OF THE PERFORANT PATH. B. Shi* and S. B. Stanfield. Lab. of Neurophysiology, NIMH, NIH Animal Center, Poolsville, MD 20853.

Axons in polymodal pathways have been shown to undergo very slow Wallerian degeneration in WLD mutant mice. It has recently been shown that in WLD mutant mice there is a delay in the intensification of acetylcholinesterase histochemical staining in the molecular layer of the dentate gyrus following lesions of the entorhinal cortex. The intensification of the acetylcholinesterase histochemical staining of septohippocampal fibres in the dentate molecular layer. Thus, it appears that delayed post-lesion reactive sprouting is associated with the delayed degeneration of cut central axons in this mutant. We have observed changes in the septohippocampal projections suggestive of sprouting of the perforant path in WLD and normal (C57BL/6J) mice by injecting wheat germ agglutinin conjugated horseradish peroxidase into the septum or commissural hippocampus and processing the tissue sections for TMB histochemistry. In normal mice changes in the distribution of labeled septal and commissural axons indicative of sprouting in the dentate molecular layer are seen as early as three days post-lesion. The earliest similar changes are found in WLD mutant mice is seven days post-lesion, when an increase in the density of labeled septal axons begins in the outer molecular layer. The delay in the sprouting of commissural axons in the mutant is even longer. Changes in the distribution of labeled commissural axons in the dentate gyrus of WLD mice are first seen twelve days post-lesion. These results confirm that post-lesion reactive axonal sprouting can be delayed in the CNS of WLD mutant mice. In addition, our results indicate that the extent that may differ among fiber systems. These findings are consistent with the notion that various CNS axonal systems may respond differently to sprouting cues and are reminiscent of the regeneration response exhibited by sensory and motor axons in the WLD mutant after peripheral nerve cuts.

DEVELOPMENTALLY REGULATED LOCALIZATION OF A PURKINJEE CELL-SPECIFIC TRANSCRIPT IN DENDRITES. E. Batenick, D. Schilling, G. Cole, S. G. and T. Oberleick, Ohio State Biochemistry Program, 2DEpartment of Cell Biology, Neurology and Anatomy, and 3The Biotechnology Center, The Ohio State University, Columbus, OH 43210. 4Abteilung Anatomie und Zellbiologie der Universität, 89069 Ulm, Germany.

Several mRNAs thought to be important for neuronal growth and plasticity have been found to be localized in dendrites. Cerebellar Purkinje cells (PCs) are ideal for studying the role such transcripts play due to both the size and monopolar orientation of their dendrites. In situ hybridization has been performed for three PC-specific transcripts known to encode proteins highly concentrated in PC dendrites. Of the mRNAs coding for these three markers, LH, PEP-19 and CaBP28K, only that for LH is abundantly detectable in PC dendrites. In addition, it appears that the amount of LH mRNA in dendrites is much greater during the early postnatal period than in adulthood, suggesting that direct translation of the LH protein in dendrites may be particularly important during dendritic outgrowth. This is consistent with several observations made of Purkinje cells in primary dissociated cultures of embryonic cerebellum. Under conditions of high K+ PC dendrites consistently take on a more elongated morphology relative to their stunted appearance in normal medium. Under the same high K+ conditions, expression of both the L7 protein and β-galactosidase driven from an L7-lacZ fusion gene, are qualitatively higher based on histological assay. In particular, in immature cultures, PC dendritic processes are L7 positive only in the high K+ condition. Thus, the L7 protein may be important for the growth and continuing plasticity of PC dendrites.

ROLE OF THE HYALURONAN RECEPTOR RHAMM IN NEURITE EXTENSION AND MOTILITY IN PRIMARY NEURONS AND NEURONAL AND GLIAL CELL LINES. U. N. Frankenstein*, J. Hacking, M. Z. Houman, R. A. Turpen* and J. J. Nagy, Department of Physiology and Laurentian Institute of Cell Biology, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba CANADA R3E 0W3.

RHAMM (Receptor for Hyaluronan Mediated Motility) has been identified as a receptor for the extracellular matrix component hyaluronan (HA) and was recently shown to be essential for the locomotion of normal and transformed peripheral cells. In view of similarities in molecular processes that govern cell locomotion and growth cone migration, we investigated whether RHAMM also contributes to cell migration of CNS-derived cells in vitro. Immunohistochemical studies of cultured primary neurons, astrocytes and oligodendrocytes as well as of several neuronal and astrocytic cell lines revealed a punctate form of RHAMM labelling in cell bodies, along processes and at growth cones. By Western blot analyses, neuronal cell lines such as PC12, NG108-15 and N5868 cells were found to express a major RHAMM forms of apparent MW of 75 and 116 kDa, while glial cells contained 50 and 72 kDa forms with HA binding capacity. Treatment of NG108-15 cells with dibrutyl-cAMP led to enhanced expression of RHAMM with a clear increase in RHAMM immunofluorescence in these cells. Monoclonal and polyclonal anti-RHAMM antibodies as well as peptides corresponding to the HA binding domains of RHAMM significantly inhibited neurite extension, glioblastoma cell extension and motility. An antibody directed against a repeat sequence present in RHAMM significantly stimulated neurite migration of NSC-14 cells and cat primary neurons. Collectively these results demonstrate the role of RHAMM in CNS cells and suggest a critical role for HA/RHAMM interactions in the modulation of neurite migration and glial cell locomotion.

We have previously reported preliminary data indicating that axon number changes at the corpus callosum in the rat. To study the callosal development, we examined the callosal axons from 15 days of age (Kim & Juraska, 1990). In adult rats, we found significant dorso-ventral and rostro-caudal variations in axonal density within the splenium, which could potentially influence axon number calculations if the splenium is not sampled extensively. Therefore, we re-examined axon number changes at the splenium at 15, 25, and 60 days of age. The posterior fifth of the corpus callosum, which contains the axons from the visual cortex at all of these ages (Kim et al., 1992), was extensively sampled with electron microscopy. Axon density was multiplied by the area of the posterior fifth from adjacent µs sections to determine axon number.

There was a significant decrease in axon number between day 15 and 25, and also between day 25 and 60. Thus, axon withdrawal in the rat splenium continues after the adult-like distribution of callosal projections within the visual cortex is established (Olavarria & Van Stuyves 1985). Preliminary results indicate that there is a sex difference in the timing of axon withdrawal in the rat splenium which will be further investigated. Supported by NSF IBN 9310945.


The BALB/cWah1 mouse strain has approximately 50% callosal defective animals. In the brains of these acellular mice, an aberrant longitudinal bundle is consistently found. This study was designed to investigate the distribution of cells and terminals of the LB and the corpus callosum (CC) during postnatal development. At ages ranging from 1-20 postnatal days (PND), 36 BALB/cWah1 mice were anaesthetized and intracardially perfused with a formaldehyde perfusate (4% PFA). Carbocyanine dyes, DiI or DiO, were inserted into the CC of normal animals or the LB of callosal defective mice. The brains were stored in 4% PFA, in darkness, for 3-7 days. After the appropriate time, each brain was embedded in gelatin and sectioned. The sections were stained with bis-benzimide and examined in an epifluorescence microscope. Analysis was focused on the paleocortex. The distribution of cells and terminals of normal and callosal defective animals (totally or partially absent CC) did not differ. At PND 5 and PND 10 axons were seen traveling through the neocortex and terminating in layer I. At PND 10 the granular layer was clearly identified and it was found devoid of callosal or LB projections. From 15-20 PND the gaps in layer IV were confirmed and the callosal and LB cells were mainly found in layers II and III. From 10-20 PND the pattern of callosal and LB projections corresponded to those portions of the parietal cortex which did not contain dense aggregates of granule cells. It was concluded that the distribution of cells and terminals of the LB resembled a normal (parallel) CC. This indicates that the appropriate targets are being located by the LB axons.

688.5 CALLOSAL AGNESIS IS SECONDARY TO DEFECTS IN HIPPOCAMPAL COMMISSURE FORMATION IN MOUSE EMBRYOS. D.J. Lively*, Dept. of Zoology, Univ. of Alberta, Edmonton, Alberta, Canada, T6G 2E9.

Pioneer axons of the corpus callosum (CC) use the hippocampal commissure (HC) as a guiding substrate to cross the telencephalic midplane in normal mice. In mice with callosal agenesis, CC axons arrive at their proper location on time to cross midplanes, but HC development is delayed, preventing its use by the CC axons as a guiding substrate. The routing and timing of hippocampal axon growth through the HC was studied in Balb/cWah1 (Wahl-50% CC defect), 129J (129-70% CC defect), and a sixth generation cross of Wahl x129 (C129/100% total CC absence) mouse embryos using perfusion fixation and the insertion of crystals of the carbocyanine dyes DiI and DiO into the hippocampal fimbria. Specimens ranged from 0.300 to 0.600g body weight or 15 to 17 days chronological age. Selected specimens were also stained using bis-benzimide to show background morphology.

In Wahl embryos, the first crossing of HC axons was seen at about 0.456g-0.48g which is about 1 day later than that seen in normal BALB/cJ hybrid embryos. In 129 embryos, first crossing was not seen until about 0.520g - 0.550g, about 2 days later than in normal hybrids. No HC axons were seen to cross midplane in the C129F, embryos prior to 0.600g. The arrival and movement of axons in all three strains appeared normal, but their traverse of midplane was blocked by the presence of a cleft which extended deep in the septal region.

Supported by NSERC grants to S.K. Malhotra and D. Wachtstein.

688.2 EARLY DEVELOPMENT OF THALAMO-CORTICAL AND GANGLIONIC EMINENCE PROJECTIONS IN THE HAMSTER. C. Méridon and P. Godelmet.* Instutut A. Fressaud, CNRS, 1 av. de la terrasse, 91190 GUYET, FRANCE.

The formation of long-range projections in the brain requires that axons navigate along precise paths and recognize their final targets. In mammals, the thalamic and the neocortical projections are connected via reciprocal connections and are separated by the ganglionic eminence. We have studied the development of these projections, from embryonic day E11 to E16 in hamster embryos, corresponding to stages when these fibers begin their growth. Small injections of carboxyfluorescein dyes (DiI, DiA) were done in fixed hamster embryo brains to label anterogradely and retrogradely the projections from small territories of the thalamus and ganglionic eminence.

Anterograde labeling shows that at E11.5, thalamic fibers reach the medial ganglionic eminence (MGE), and cortical fibers reach the lateral GEM. Importantly, retrogradely-labeled neurons are found in close proximity to the growth cones of thalamic and cortical fibers in the medial and lateral GEM, respectively. Thus, at this age, there are reciprocal connections between the GEM and the thalamus and neocortex. Injection into the ganglionic eminence confirms the existence of this early projection. At E12.5, thalamic fibers accumulate at the frontier between the lateral GEM and the neocortex. They cross into the intermediate zone of the neopallium one day later, at E13.5. The most advanced reach the dorso-medial part of the parietal subplate and few or no fibers are found in more rostral or caudal levels. From E11.5 to E12.5, cortical fibers grow forward and into the GEM. At the frontier between the neocortex and the GEM, they make a right-angle turn. The first cortical fibers reach the dorsal thalamus at E13.5. At this age, retrogradely labeled cells are observed in the lateral part of the parietal cortical plate, and in the ventro-basal region of the dorsal thalamus, suggesting that these regions are the first that establish reciprocal connections.

These observations show that thalamic and cortical fibers project to the ganglionic eminence before reaching their targets. At early stages, the GE interconnects the diencephalic and neocortical regions, and therefore, interactions of thalamic and cortical fibers with the GEM or its projections could be involved in the establishment of thalamo-cortical connectivity during early development.

688.4 PEP-19 IMMUNOISTOCHEMISTRY REVEALS THE DEVELOPING DIRECT PURKINE-VESTIBULAR COMPLEX PROJECTION IN THE RAT. J. J. Viele F. B. Miller and W. V. Young. Dept. of Pathology (Division of Neuropathology). 2Anatomy and Cell Biology, and the Brain Research Inst., UCLA, Los Angeles, CA 90024.

We performed an immunohistochemical examination of the developing direct purkinje vestibular complex projection in the rat cerebellum. The antibody utilized to discriminate Purkinje cell axons from others within the developing metencephalon was a rabbit polyclonal antibody produced against the Purkinje cell enriched antigen, PEP-19. In mouse, we identified 6.2 kDa peptide containing regions of similarity to a number of proteins thought to interact with calcium, including S100 calcium and calmodulin. A previous immunohistochemical examination of PEP-19 in the adult rat showed immunolocalization to corticopontine neurons and a minor proportion of neurons within the dorsal cochlear nucleus. The earliest age we have examined embryonic 17 (E17), shows no immunopositive axonal profiles within the vestibular complex (superior and lateral vestibular nucleus). At E20, well organized fascicules are seen passing through the deep cerebellar nuclei and the pontocerebellar isthmus. These axons are observed to enter into the vestibular complex and apparent synaptic contacts upon cells of all stags within the lateral vestibular nucleus are seen. From these initial findings we conclude that the direct purkinje vestibular complex projection is established between E17 and E20.

688.6 MULTIPLE GUIDANCE CUES ARE INVOLVED IN THE SEGREGATION OF PIONEERING PATHWAYS IN THE CORTICAL AND INTERNAL CAPSULE: EVIDENCE FROM REELE R.A. Kindeys*, W. Wang, A. Sharma Dept. of Neurology, SUNY at Stony Brook, Stony Brook, NY 11794.

We compared pathway formation during cortical development in normal and reeler mutant mice using the carbocyanine dyes DiI and DiA to trace axons in fixed tissue, and immunolabelling with antibodies to chondroitin sulfate proteoglycans (CSPGs) to identify the extracellular matrix (ECM). By embryonic day E11, neurons begin extending axons from the preplate zone (PPZ) which has intense CSPG labeling. By E12, cortical plate (CP) neurons begin splitting the PPZ into the marginal zone (MZ) and subplate (SP), which retain CSPG labeling. CP neurons pass through the SP region and obliquely cross the intermediate zone (IZ), exiting the cortical close to the VZ. Reeler neurons retain the PPZ, and their IZ and CP are mixed in one layer. Axons of CP neurons extend obliquely along this layer and exit the VZ. By birth, normal and reeler, pioneer efferents hesitate in a zone above the forming stratum. If later arriving (E12-13) thalamic axons encounter the waffling efferents, they fasciculate. The time period of co-extension is brief. In normal neocortex, thalamic axons can selectively extend within the subplate and its CSGP. As the stratum matures, thalamic axons extend laterally around expanding stratum, to enter the subplate and its CSGP. CP axons continue to exit the cortex near the VZ. The CP axons are globally distributed and separate and axons do not encounter each other after E14. Reeler E14 'CP' develops streaks of CSGP intermingled with efferent neurons and axons allowing reeler thalamic afferents to encounter cortical thalamic axons. Thalamic axons selectively extend within the streaks of CSGP, and thalamic axons. Evidence indicates that thalamic and cortical axons guide each other in development, it occurs in a limited number of axons. Multiple guidance cues produce pathway segregation in the forming cortex and internal capsule.
688.7 POTENTIAL ROLES OF THE CCK5 AND CCK8 RECEPTOR TYROSINE KINASES IN THE DEVELOPMENT OF NERVOUS SYSTEM ARCHITECTURE. L. A. Rojalst and F. P. Pascual, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Many receptor-tyrosine kinases have been shown to play important roles in nervous system development. We are currently probing the effects of extracellular "growth factors" with neural activity. The Eph subclass represents a major branch of receptor tyrosine kinases that are highly expressed in the developing nervous system. We have shown that the spatial distribution of two Eph-related kinases, CCK5 and CCK8, in the embryonic peripheral nervous system and brain is different. At HH stages 10 and 18, both CCK5 and CCK8 appear to be expressed in outgrowing motor axons. High levels of phosphorylated immunoreactivity correspond to the sites of CCK5 and CCK8 axon growth cones. At HH stages 10 and 18, the axon growth cone becomes restricted to a subset of spinal neurons, whereas CCK5 is expressed throughout the spinal nerves. By 8 days in ovo, CCK8 expression in peripheral nerves decreases, but appears high peripherally, whereas CCK5 expression remains high within the nerve trunks. CCK5 and CCK8 are also expressed in the developing visual system. The pattern of CCK8 expression suggests that it plays a role in the formation of maintenance of the ganglion cell pathway from retina to optic tectum. CCK8 is expressed in the retina itself and becomes restricted to ganglion cell somata. By 8 days in ovo, CCK8 expression is apparent throughout the optic nerve, and by 10 days in ovo, CCK8 immunoreactivity outlines the optic tract. CCK5 appears to play a role in the development of retino-tectal specificity. It is highly expressed in the ventral portions of the developing retina and optic nerve, but only weakly expressed in the dorsal portions of these structures. Immunostaining of retinal explants demonstrates that CCK5, CCK8 and phosphorysine immunoreactivities can be linked to neuronal processes. This work was supported by NIH Grant HD26351.

688.8 CORTICOSPINAL TRACT IN THE DEVELOPING RAT SPINAL CORD: THE PARENT AXONS AND PROJECTION COLLATERALS. J. Hagesater* and J. Zent, Department of Anatomy, Hokkaido University School of Medicine, Sapporo 060, Japan.

A detailed morphological analysis of the corticospinal tract of the rat neonate ranging from postnatal day 1 (P1) to P14 was investigated using retrograde labeling with fluorescent dye, DII, optimized by confocal laser scanning microscopy (CLSM). The parent axons constituted a compact bundle which was constantly enclosed in the ventral part of the dorsal funiculus of the spinal cord. Individual axons were not parallel to each other within the bundle. In addition, the growth cones of follower axons were distributed over a wide range. The majority of the projection collaterals toward the target area emanated from the rectangular branching, however, a number of them ran in various directions. Multiple collaterals from the single parent axon were often observed. The projection fibers repeated branching and their arbors terminated onto the preferential phenotype of the target neurons within the dorsal horn, the intermediate zone or the ventral horn in the spinal gray matter.

688.9 AXONAL ARBORIZATIONS OF THE PRIMARY AFFERENTS SERVING DIFFERENT VESTIBULAR END-ORGANS IN ZEBRAFISH EMBRYOS. S.E. Fraser* and R. Chapman, Division of Biology, 139-74, Caltech, Pasadena, CA 91125.

In adult vertebrates, primary vestibular afferents serving the different semi-circular canal and otolith organs form distinct overlapping regions of axonal arborization in the vestibular nuclei. While there is an obvious role for this specificity in sensory processing, little is known about the development mechanisms which are responsible for establishing the connections. In particular the relative contributions of inherent positional cues, topographic, and patterns of neural activity to the development of specificity have not been previously examined. As a first step in answering these questions we have studied the axonal arborization patterns of primary vestibular afferents in zebrafish embryos. To achieve this we have used iontophoretic injections of lipophilic dyes into the crista and maculae in the inner ears of fixed zebrafish embryos. At HH stages, we inject dyes to different lobes of the sacculus and in the utricle we examine the patterns of arborization in area octavolateralis in the hindbrain using confocal microscopy of whole mounts of the labeled embryos. We find that the primary afferents serving the different vestibular end-organs have recognizably different projection patterns. The pattern of axonal arborization is different from one given end-organ, however, it is highly stereotyped and reproducible between animals at a given age. Double labeling experiments using Dil (C3) and Dii (C8) injected into different cranial nerve in the same animal have shown that there is good separation of the arbors of the populations of axons serving different end-organs.

Further experiments are currently underway to test whether the development of normal patterns of axonal arborization in the vestibular system depend on the presence of normal neural activity, as has previously been shown to be true in the visual system.

688.10 AXONOGENESIS AND THE EARLY APPEARANCE OF GABA IMMUNOREACTIVE NERVES DURING DEVELOPMENT OF THE CNS IN A TELEOST. I. Olsén* and F. Erikson, Dept. of Zoology, Univ. of Lund, Sweden.

During early CNS development, a small number of neurons give rise to pioneer axons that form the axonal scaffold. It is not known whether these axons acquire neurotransmitter phenotype, or which transmitter(s) they utilize. In an attempt to approach this question we have studied the early development of the axonal scaffold in embryos of a teleost fish, the three spined stickleback, with immunocytochemical techniques. We used mono- clonal antibodies against acetylcholinesterase (AChE) and against GABA. GABA is known to exert various neurotransrophic actions in the developing nervous system. The antibodies were applied to the semithin Araldite sections and to whole mount embryos. Both single and double labeling experiments were performed. Huntingdon neuroblasts of 144-168 post fertilization (PF). At 36 PF, two 6-11B-1-immunoreactive (6-11B-1) ventral tracts are located in the mesencephalon. At 48 PF, two 6-11B-1 ventral tracts extend from the mesencephalon throughout the spinal cord. At 96 PF, the first GABA-immunoreactive (GABA) neurons appear in the ventral mesencephalon, ventral mesencephalon and in the spinal cord. At 600 PF, the 6-11B-1 ventral tracts, were connected by numerous commissures all along between the mesencephalon and the spinal cord. Also, an additional lateral ventral tract appeared. At 66 PF, the first GABAergic cell groups have grown by addition of GABAergic neurons, and GABAergic cell bodies and fibers are closely associated with the 6-11B-1 tracts that constitute the axonal scaffold. Thus, GABAergic neurons appear only when the axonal scaffold is established. We suggest that the first pioneer neurons are not GABAergic but that GABAergic neurons make an important contribution to the formation of the early nervous network.

688.11 COMMISURE FORMATION IN THE EMBRYONIC BRAIN OF THE GRASSHOPPER, G. BOSYERI, AND H. Reichert, Zoology Institute, University of Basel, Rheinsprung 9, CH-4051 Basel, Switzerland

A fundamental event in the normal development of the insect brain is the establishment of bilateral commissural connections between the brain hemispheres. We have studied the formation of the very first commissural fiber pathways in the embryonic grasshopper brain using immunocytochemical techniques. During early embryogenesis a set of approximately 130 neuroblasts differentiates from the neuroepithelium of the head to form the bilaterally organized proto-, deut- and tritocerebral brain segments. In the midline of the deutocerebrum, however, an additional set of 15 neuroblasts differentiates from the neuroepithelium anterior to the stomodaeum, and ventrally between the pars intercerebralis lobes of the protocerebral segment. We have identified these neuroblasts as a group of cell falks that give rise to a midline intercerebral bridge. The differentiation of this brain commissure depends on the axoloc in gene. In axoloc in mutants the brain commissure is severely reduced, and only a small subset of commissural falks are formed. The cerebral hemispheres are linked to the ventral nerve cord by paired brain connectives, which interconnect the initially separated prosophageal and ventral neurogenic regions. The formation of these pair brain connectives depend on the axoloc in gene. In axoloc in mutants paired connectives do not form, and the aberrant ectopic midline ventral nerve cord is established by axons which navigate along the midline stomatostomaic nervous system structures. Our future goal is to carry out a comprehensive study of the formation of fibers connecting the brain segments involved in brain development. (Supported by SNSF and HHMI).

688.12 MOLECULAR MECHANISMS OF EMBRYONIC BRAIN DEVELOPMENT IN DROSOPHILA MELANOGASTER. S. Thienast, S. Leurzi, F. Hirth, C.S. Goodman and H. Reichert, Dep. of Zoology, University of Basel, CH-4051 Basel, and HHMI, Dep. of Molecular and Cell Biology, University of California, Berkeley, California 94720.

We are using the powerful genetic and molecular genetic techniques that are available for the study of embryonic development in Drosophila to investigate the molecular mechanisms involved in building a complex brain. Embryonic development of the Drosophila brain is comparable to other insects. During the period of embryogenesis in Drosophila, complex cerebral hemispheres are formed. These cerebral hemispheres are interconnected by the brain commissures, which is provided by a pair of axoloc in cell falks that give rise to a midline intercerebral bridge. The differentiation of this brain commissure depends on the axoloc in gene. In axoloc in mutants the brain commissure is severely reduced, and only a small subset of commissural falks are formed. The cerebral hemispheres are linked to the ventral nerve cord by paired brain connectives, which interconnect the initially separated prosophageal and ventral neurogenic regions. The formation of these paired brain connectives depend on the axoloc in gene. In axoloc in mutants paired connectives do not form, and the aberrant ectopic midline ventral nerve cord is established by axons which navigate along the midline stomatostomaic nervous system structures. Our future goal is to carry out a comprehensive study of the formation of fibers connecting the brain segments involved in brain development. (Supported by SNSF and HHMI).
688.13

ESTABLISHING FUNCTIONAL RELATIONSHIPS BETWEEN GENES IN AB1-REGULATED PROCESS ES BY ANALYSIS OF DROSOPHILA MUTANT PHENOTYPES. J.-L. Luang*, F. M. Hoffmann, McArdle Laboratory 77for Cancer Research, University of Wisconsin-Madison, Madison, WI 53706.

The function of the Ab1 protein tyrosine kinase in Drosophila is of interest because it relates to the transforming oncogene in Abelson murine leukemia virus. Drosophila provides a well-characterized system in which to analyze the developmental consequences of specific mutations by examining mutant phenotypes. Five genes, disabled (db), enabled (en), faulx axon connections (facc), fasciclin I (fasci) and fasciclin II (fascii) have been examined to analyze their functional interaction with the Ab1.

We have observed defects in axon outgrowth and/or pathfinding in embryonic CNS of these mutants. We also used enhancer trap lines to visualize a small subset of axons. Our preliminary data suggest the axon tract defects observed in mutant embryonic CNS are due to defects in cell adhesion mechanisms critical to growth cone pathfinding. Antibody staining of CNS-derived cell lines have indicated that the abs, db and ena proteins are present in the neuronal cytoplasm, neurites, and the growth cone regions.

688.15


Corticothalamic fibres undergo extensive reorganisation between the cortex and the thalamus. To examine this reorganisation as connections are being formed, we placed small crystals of the lipophilic dyes DiI and DiA in the cortex of acutely or chronically decerebrated rats. Two days later, neurites were visualized immunohistochemically and counted. The number of neurites on the bottom surface of the membrane was increased 2 to 5 fold by the explant conditioned medium compared to normal unconditioned medium. NT-3 containing medium resulted in a 10 fold increase in the number of neurites on the bottom surface of the membrane. The NT-3 effect is completely abolished when the gradient is eliminated by adding NT-3 containing medium to both the upper and lower chambers, suggesting that the effect is due to chemotaxis and not simply enhanced neurite outgrowth. Experiments are in progress to determine if corticofugal neurites are among the NT-3 responsive population and whether these neurones express trkC in situ.

689.1


Two distinct types of acetylcholine receptor (AChR) channels are present at the synaptic membrane of vertebrate skeletal muscle. One type is characterized by high conductive (HC) fast kinetics, and the other type is characterized by low conductance (LC) slow kinetics. In an effort to define the role of innervation in the expression of these two classes of AChR channels we performed current-clamp recordings in the motoneuronal pool of adult mice. This was followed by sequential single channel recordings performed in freshly dissociated fibers from the flexor digitorum muscle. Up until two days after crush the percentage of LC channels were less than 1% of the total observed events and similar to the control. At three days post crush the percentage of LC channels started to rise, and by day five more than 40% of the channels were LC type. This was followed by a progressive decrease of the percentage of LC type, and by eight days post crush less than 40% of the openings were LC type. We concluded that the whole cell patch clamp technique is important to regulate the expression of either type of channels and that single channel recordings are useful to monitor the course of reinnervation.

689.2

DECREASED ACTIVITY OF Na CHANNELS IN PITUITARY MELANOTROPHS FOLLOWING THE ONSET OF DOPAMINERGIC INNERRATION. J.C. Gorona, A. Navarrete, A. Marin and C. Costa. Dept. of Neurosciences, Cinvestav, Mexico, DF 07000.

High-threshold Ca** currents in rat melanotropes undergo a drastic inhibition during the early postnatal period, concomitant with the onset of dopaminergic innervation (Gorona et al., Soc. Neurosci. Abstr. 19:1128, 1993). We have now investigated whether the long-term effects of innervation include regulation of Na current. Na currents were dissociated from non- and fully-innervated rat intermediate pituitary lobes (postnatal 0-4 days, 4-8 days, and 11 days, respectively). In the glass culture for 5-24 h, and then subjected to whole cell patch clamp. Standard recording solutions were used. Peak Na** inward current decreased to -54 +54 pA (mean±SE, n=14) in PNL cells to -28±14 pA (n=14) in PMI1 cells. In contrast, the average values for cell capacitance and K+ outward current amplitude did not significantly differ between these two types of cells. Thus, the dopaminergic innervation of melanotropes reduces Na** current density, but does not seem to affect the long-term activity of K+ channels.

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FUNCTIONAL EXPRESSION OF Ca<sup>2+</sup>-ACTIVATED K<sup>+</sup> CURRENTS IN EMBRYONIC CHICK SYMPATHETIC NEURONS DEVELOPING IN SITU AND IN VITRO. M. E. Waisberg, S. Raucher, and S. E. Dryer. Program in Neuroscience, Florida State University, Tallahassee, FL 32306

We have examined the expression of whole-cell K<sub>Ca</sub> in embryonic chick sympathetic neurons developing in situ and in vitro. K<sub>Ca</sub> expression appears related in vivo to the expression in developmental E13 and reaches its mature density at E18. Voltage-activated Ca<sup>2+</sup> currents (I<sub>Ca</sub>) are present throughout these stages. When sympathetic neurons are isolated at E13 and maintained in cell culture for an additional five days, both they do not express K<sub>Ca</sub> although I<sub>Ca</sub> is expressed at normal density. Culturing sympathetic neurons in the presence of 5 ng/ml NGF caused a significant (p < 0.05) increase in K<sub>Ca</sub> expression, although this effect appeared to be restricted to a subpopulation of cells (17 out of 34 neurons). Similarly, co-culture of sympathetic neurons with ventricular myocytes stimulated K<sub>Ca</sub> expression (p < 0.05 compared to controls). By contrast, co-culture of sympathetic neurons with spinal cord explants, which make functional contacts with sympathetic cells, did not promote K<sub>Ca</sub> expression. The developmental expression of K<sub>Ca</sub> is dependent upon extrinsic factors, possibly including target-derived trophic factors. Supported by NIH Grant NS-27013.

OTHER FACTORS AND TROPHIC AGENTS I

Localization of Rse, a Novel Tyrosine Kinase Receptor, in the Mammalian CNS; Comparison with Other Tyrosine Kinase Receptors. Mark Amundson, Lanly Wong, Klaus Becker, Franz Hell. Neurobiology, University of Toronto, Toronto, ON, Canada.

Rse is a novel tyrosine kinase receptor isolated from rat and human brain. Rse and human Rse share ~90% amino acid identity, containing extracellular, transmembrane, and intracellular domains. Recent analysis reveals Rse expression to be most abundant in brain. Here, in-situ hybridization was performed to identify specific neuronal subcategories expressing this gene.

In-situ hybridization revealed Rse to be widely expressed throughout the adult rat brain, but restricted to specific regions. Expression in the hippocampus was observed primarily in CA1, to a much lesser extent in CA3 and CA4, and undetectable in the dentate gyrus. High levels of expression were observed in the cortex, cingulate, olfactory bulb, and cerebellar granule cells, but undetectable in the midbrain, brainstem, and medial septum. Sections from the rat hippocampus also revealed high levels of Rse expression in these structures. In the E15.5 mouse and 8 week human embryo, expression of Rse was undetectable in the CNS. Thus, expression appears to be turned on later in development, with highest levels being maintained in the adult. The regulation of Rse expression in response to a combination of nerve-forming/interventricular lesion was also investigated.

The localization, developmental patterns, and response of Rse expression to injury were compared with other recently described tyrosine kinase receptors of the mammalian CNS.


The protein kinase inhibitor K-252a has been shown to promote cholinergic activity in cultures of rat spinal cord (Glickman et al., J. Neurochem., 61: 210-221, 1993) and neuronal survival in chick dorsal root ganglion cultures (Lorincz, et al., Neurosci. Lett. 108: 207-212, 1990). To determine the mechanism by which K-252a acts as a neurotrophic factor, we examined the effects of this molecule on a human neuroblastoma cell line, SH-SY5Y. K-252a induced neurite outgrowth in a dose-dependent manner. Co-incubation with neurotrophin-3 (NTS) enhanced K-252a-mediated induction of 125 and 140 kDa tyrosine phosphorylated proteins. Inhibition of protein kinase C with GF-109203X did not prevent K-252a induced tyrosine phosphorylation or K-252a dependent neurite outgrowth suggesting that the neurotrophic effects mediated by K-252a are independent of protein kinase C inhibition. We have identified one of the phosphoantibodies as the p815 focal from the cerebellum and dorsal-teal prefrontal cortex were obtained with increased Fak activity and appeared to be independent of ligand/intergen interaction. The induction of Fak phosphorylation, by K-252a, was also observed in SH-SY5Y and primary cultured dorsal root ganglion cells but not in PC12 cells. The protein kinase-C independent induction of tyrosine phosphorylation and the identification of Fak as a substrate of K-252a-induced tyrosine kinase activity suggests that this compound mediates neurotrophic effects through a novel signaling pathway.
LPA, A NEURITE OUTGROWTH INHIBITOR, ACTIVATES MAP KINASE IN NIE-115 NEUROBLASTOMA CELLS. G.L. Ramakers* and W.H. Moolenaar. Division of Cellular Biochemistry, Netherlands Cancer Institute, Plesmanlaan 121, Amsterdam, The Netherlands.

The phospholipid lysophosphatic acid (LPA) has recently been identified as an albumin-bound serum factor, that induces DNA synthesis and stress fiber formation in fibroblasts by activating both classic and novel signaling pathways through a putative 40-kDa G-protein receptor coupled to the heterotrigic G-proteins (Moolenaar, Trends Cell Biol. 4: 213; 1994). The 40 kD LPA receptor is highly expressed in brain and in the neuroblastoma cell line NIE-115, which differentiates to a sympathetic phenotype upon serum withdrawal. In differentiated NIE-115 cells LPA induces rapid neurite retraction through an independent signal pathway involving classic second messengers (Jalink et al., Cell Growth Differ. 4: 247; 1993). As protein tyrosine kinase and phosphatase inhibitors interfere with LPA-induced neurite retraction in NIE-115 cells, we investigated protein tyrosine phosphorylation in NIE-115 cells by LPA after serum-starvation, using an anti-phosphotyrosine immunoblotting.

LPA (at 100 nM and higher), thrombin and insulin, but not EGF, EGF or glutamate transiently stimulated the phosphorylation of p42 MAP kinase with a peak at 2 min. LPA-induced MAPK phosphorylation was inhibited by pertussis toxin and various suppressors of protein kinase (PKC), indicating an involvement of the heterotrigic G-protein, as well as PKC. A model will be presented in which LPA-induced MAP kinase parallels, but is independent of neurite retraction, and serves as a trigger to drive NIE-115 cells back into the cell cycle.

**690.5**


The distribution of GAP-43-like immunoreactivity (LI) in the superior cervical ganglia and irides of adult SD rats was studied using confocal laser scanning microscopy (CLSM). In the SCG of control rats, GAP-43-LI was mainly located in the nerve terminals around, but not inside, the principal neurons and very little in nerve bundles. After 24 hours decentralization the GAP-43 positive nerve terminals disappeared, while the principal neurons remained. The TR-LI positive nerve terminals 3 days after decentralization, GAP-43-LI was again observed in nerve terminals, and also appeared in axon bundles, while principal neurons disappeared. Principal neurons in SCG cells did not show clear changes after decentralization. In the irides of control rats, GAP-43-LI was present and distributed in a patchy pattern in axon bundles, around blood vessels and in the terminal network. Double immunostaining showed that GAP-43-LI colocalized with TH-LI, but appeared to distribute differently from SP-LI. GAP-43-LI semi-quantification of GAP-43-LI in the irides was performed using CLSM. The intensity and density of the nerve terminal network in the dilator were registered. 3 days of decentralization, the intensity and density of the GAP-43-LI positive terminal network significantly increased.

The results indicate that GAP-43-LI is normally present in nerve fibers and terminals of the post-ganglionic adrenergic SCG neurons in adult rats. The modulations in GAP-43-LI observed in cell bodies and nerve terminals after decentralisation suggests that these autonomic neurons have a capacity for remodelling and plasticity which may partly be regulated via the pre-ganglionic neurons.

**690.6**

IDENTIFICATION OF INTERLEUKIN-6-EXPRESSING NEURONS IN DIFFERENT REGIONS OF RAT BRAIN. R. A. Gaden* and U.H. Oomen, Dept. Physiology, Univ.Basel, CH-4051 Basel, Switzerland

Interleukin-6 (IL-6) belongs to a family of neurotrophic cytokines which includes ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M and IL-11. This family of multifunctional proteins shares a common signal-transducing receptor subunit (gp130). We focused on IL-6, which promotes neuronal survival, differentiation and ameliorates the effects of excitotoxins. In a recent study using reverse transcription-polymerase chain reaction (RT-PCR) we showed that both IL-6 and IL-6 receptor (gp80) transcripts are found in normal rat brain in a region-specific manner and are developmentally regulated. To identify the cellular origin of IL-6 and its receptor, we established a sensitive non-radioactive in situ hybridization technique. In line with our RT-PCR data the most prominent IL-6 mRNA expression was observed in hippocampus and hypothalamus. Low expression was seen in the striatum. The IL-6 mRNA signal was predominantly located in neuronal cells, such as the pyramidal and granular cells of the hippocampus and the Purkinje cells of the cerebellum. These findings implicate IL-6 in the regulation of so far unidentified neuronal functions. This study was supported by the Swiss National Foundation for Scientific Research (Grant 31-39129.93) and by the BMTF (Grant 01KL9303).

**690.7**

INTERLEUKIN-18 EXPRESSION DURING DEVELOPMENT OF THE MOUSE BRAIN. J.L. Scicpiter* and C.F. Ide, Neuroscience Training Program and Department of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118

Interleukin-18 (IL-18) is a cytokine which modulates inflammation and immune responses in the periphery. Expression of IL-18 has been demonstrated in the adult brain after trauma and may be regulated during cell death. Since development of the brain involves programmed cell death, we investigated the developmental profile of IL-18 immunoreactivity (ir) in the perinatal mouse brain using antiseraum to human recombinant IL-18. Immunohistochemistry on E10, P0, P6 and P30 animals. We also synthesized a 447 bp cDNA probe (to murine IL-18 mRNA) for use in in situ hybridization studies on E10, E15, E18, P0, P6, P10, P20 and P60. Antisense and sense probes were used to detect IL-18 mRNA in situ. The mRNA was found in cells of all brain areas, especially in the cerebral cortex. At P6, mRNA levels declined and IL-18 was equivalent to levels and areas found at P0 and P10. IL-18 mRNA declined in all brain regions except the olfactory bulb and the CA1 region of the hippocampus. In adults there was no detectable IL-18 mRNA except in the olfactory bulb and the CA1 region of the hippocampus. IL-18 mRNA was only faintly expressed and was restricted to the olfactory bulb and the CA1 region of the hippocampus. This suggests that IL-18 is downregulated during natural cell death in the developing CNS. Supported by DOD grant #2-89/Y116/SS-150 to the Tulane/Xavier Center for Bioenvironmental Research.
690.9 EFFECTS OF ENDOTHELIN-I ON THE EARLY DEVELOPMENT OF CHICK EMBRYOS. L. Hess* and A.Y. Jeng, Department of Biology, Seton Hall University, South Orange, NJ 07079 and Research Department, Clinical Pharmaceuticals, Summit, NJ 07901. Endothelin-I (ET-1) is a potent vasoconstrictive peptide originally isolated from conditioned medium of porcine aortic endothelial cells. We have previously found that ET-1 enhances neurite outgrowth from phorbol ester-treated dorsal root ganglion explants. The purpose of the present study is to examine the effect of ET-1 treatment on the development of the nervous system in chick embryos. Eggs were incubated at 37°C and treated with ET-1 (50 pmol) through a 24-hour period. Repeated treatment with 50 pmol ET-1 from days 2-4 resulted in significantly larger heads and trunks when observed on day 7, while the size of the eyes and limbs were comparable to the controls. The overall lengths of the diencephalon and the telencephalic and optic lobes in the treated groups were increased. Similar effects on brain development were noted when embryos were treated with a single dose of 50 pmol ET-1 on day 5. These results suggest that ET-1 has growth-factor-like effects on both development and that embryos are more responsive to treatments around day 5.

690.10 RELATIONSHIP OF c-neu ONCOGENES AND THE EPIDEMICAL GROWTH FACTOR RECEPTOR (EGFR) IN DEVELOPING RAT CEREBRAL CORTEX. P.E. Kuhn* and M.W. Miller, Cell and Devol. Biol., Rutgers Univer., Piscataway NJ 08854, Res. Serv., V.A.M.C., Iowa City IA 52242, and Depts. of Psychiatry and Pharmacology, Univ. of Iowa Coll. of Med., Iowa City IA 52242. Similarities between c-neu oncoproteins and EGFR were examined with antibodies directed against the carboxyl ends of the oncoprotein and the EGFR. During the first two postnatal weeks, both antibodies identified neural cell bodies distinctly throughout cortex. Hence, both proteins may be interrelated. The transient pattern of oncoprotein immunostaining correlated with the transient expression of a 25 and a 300 kDa protein. The anti-EGF, identified a 50 kDa protein which was expressed transiently during the first two postnatal weeks. After immunoprecipitating cortical tissue with the anti-oncoprotein antibody, evidence of the EGFR + 50 kDa protein in the supernatant was eliminated; the precipitate retained all of the immunolabeling. In the reverse experiment, tissue was immunoprecipitated with the anti-EGF; the 35 and 300 kDa proteins remained unprecipitated in the supernatant. Immunoprecipitation with either antibody did not affect detection of the oncoprotein + or EGFR + proteins with different profiles of temporal expression. The EGFR + 50 kDa protein shares an epitope with a c-neu oncoprotein(s). Thus, the 50 kDa protein (a) forms a heterodimer with a c-neu oncoprotein or (b) is a component of a larger c-neu oncoprotein (~ 300 kDa protein). Supported by the V.A. and N.I.H. (DE07734, AA06916, and AA07568).

690.11 LOCALIZATION OF RETINOIC-BINDING PROTEINS AND RETINOID RECEPTORS SUGGESTS A FUNCTION FOR RETINOIC ACID IN DEVELOPING AND ADULT Dopamine-Nerve TERMINAL BRAIN AREAS. E.H. Egestad,* A. Tomas,* E. Eriksson,* and R. Nishi, Department of Cell Biology and Anatomy and Department of Biological Structure and Function, Oregon Health Sciences University, Portland, OR 97239. The choroidal nerve of the avian ciliary ganglion innervate the smooth muscle cells surrounding the arterial vasculature of the choroidal layer of the eye. These neurons are cholinergic and express somatostatin as a neuropeptide. When grown in culture, the smooth muscle cells secrete Actinin A (AA), which has been shown to increase the expression of somatostatin in the neurons of the ciliary ganglion. We have characterized the target cells to determine expression of smooth muscle markers to assess culture time. The cells lose expression of smooth muscle specific actin (SMA) when placed in culture, but redifferentiate over time. Treatment with ET-1 increases proliferation of the cells while suppressing the expression of muscle markers. In contrast, TGFβ rapidly increases expression of SMA while suppressing proliferation. We have used ET-1/PCR to isolate and clone a fragment of AA from E12 choroid, showing that AA is present in vivo, as well as being expressed in vitro. We have initiated studies to examine the in vivo expression of AA expression during choroidal smooth muscle cell differentiation in culture in addition to parallel studies of AA expression in the choroid layer in vivo. Supported by NS25767 and NIHES23207123.

690.12 DEVELOPMENTAL INTERACTIONS BETWEEN CHOROID NEURONS AND THEIR SMOOTH MUSCLE TARGET CELLS. D.C. Durand, G.G. Leblanc,* and R. Nishi, Department of Cell Biology and Anatomy and Department of Biological Structure and Function, Oregon Health Sciences University, Portland, OR 97239. We have characterized the target cells to determine expression of smooth muscle markers to assess culture time. The cells lose expression of smooth muscle specific actin (SMA) when placed in culture, but redifferentiate over time. Treatment with ET-1 increases proliferation of the cells while suppressing the expression of muscle markers. In contrast, TGFβ rapidly increases expression of SMA while suppressing proliferation. We have used ET-1/PCR to isolate and clone a fragment of AA from E12 choroid, showing that AA is present in vivo, as well as being expressed in vitro. We have initiated studies to examine the in vivo expression of AA expression during choroidal smooth muscle cell differentiation in culture in addition to parallel studies of AA expression in the choroid layer in vivo. Supported by NS25767 and NIHES23207123.

690.13 CHARACTERIZATION OF SOLUBLE FACTOR ACTIVITIES IN DEVELOPING CHICK CILIARY GANGLION - IRIS SYSTEM. B.A. Link, W.K. Woodworth, and R. Nishi, Dept. of Cell Biology and Anatomy, Oregon Health Sciences Univ., Portland OR 97201. The differentiation of the developing iris and innervation by ciliary ganglion neurons is an excellent system to study neurite target interactions. The developing chick iris is composed of two muscle groups, the myoepithelial or smooth-type muscle constrictor and the striated-type muscle dilator. Mesoepithelial cells are believed to further differentiate to the striated-type muscle and give rise to the contractile dilator at E13. Previous studies (Link and Pilat, 1974 J. Physiol 241:77) have shown that the iris is initially innervated by the ciliary ganglion and that innervation with innervation of the iris is a period of cell death in the ciliary ganglion which results in a 50% loss of the neurons. To investigate potential retrograde influences, we have used immunoperoxidase and immunoperoxidase to identify soluble activities from the ciliary ganglion and the iris. Soluble media from E9 to E11 iris were prepared and applied to a heparin agarose column. Heparin binding and non-binding fractions were tested for [3H]thyrosine incorporation into murine AKR-2B cells in the presence or absence of heparin. Heparin neurotrophic activity (c-like, a-like) was identified by its ability to support neurite outgrowth of cultured E8 ciliary ganglion (CG) neurons. Soluble extracts from E9 to E14 CGs were assayed in a transgenic assay system. Extracts of iris tissue we observed developmental increases in intraneuronal activity with the greatest activity in the heparin binding - heparin dependent fraction. We observed intraneuronal activity to further develop to the heparin dependent - heparin dependent and non-heparin dependent binding fractions. As the developing iris, heparin binding - heparin dependent and non-heparin binding fraction were observed. These data suggest that heparin binding activity may play a role in the development of the iris and ciliary ganglion. Supported by NS25767.

690.14 Transforming Growth Factor alpha immunoreactive nerve fibers in the rat and mouse intestinal tract. P. Hoffmann, J. Lachman, J.M. Zeeh, L. Liu, M. Sottelli, L. Barajas, M.G. Ercis*, and V.E. Ryszewski. Harbor UCLA Medical Center, Torrance, CA 90509. Epidermal Growth Factor (EGF) and Transforming Growth Factor (TGFα) are important mediators of mucosal blood flow, mucus production and secretion, and motility in gastrointestinal tissue in addition to their mitogenic actions. These effects are important since they may mediate EGF/TGFα’s capability to protect intestinal mucosa against acute injury. The aim of the present study was to show morphological evidence of the invasion of TGFα in the regulation of the enteric nervous system by demonstrating TGFα immunoreactivity in nerve fibers of stomach and colon in rats and mice. Tissue samples were fixed in Zamboni’s solution, frozen in OCT compound and cut with a cryostat. A polyclonal antibody, raised against conjugated rat TGFα, was used to perform immunohistochemistry. Binding was visualized by the avidin-biotin peroxidase technique as described previously. Beaded nerve fibers were detected in the muscle and submucosal layers of stomach and colon in rats and mice. A weak immunoreactivity was also detected in neurons of the myenteric plexus of both species. Detection of TGFα immunoreactivity in neural structures of intestinal tissues gives morphological evidence that TGFα released from these nerve fibers may be the endogenous source to induce EGF/TGFα’s nonmitogenic actions in vivo.
THE EFFECTS OF TRANSFORMING GROWTH FACTOR-β (TGF-β) ON NEURONAL AND GLIAL CELLS ON THE DEVELOPING MEDIAL SEPTAL AREA. I. E. Menn et al, Centre de Recherche Pediatrics-Neuro Scie, Montreal, Quebec, CANADA, H3T 1C5.

We have examined the effects of TGF-β on different neuronal and glial cell populations in primary cultures of dissociated fetal rat medial septal cells. TGF-β, like epidermal growth factor (EGF), was found to induce a dose-dependent increase in choline acetyltransferase (ChAT) activity which was accompanied by a parallel decrease in the number of acetylcholinesterase positive neurons. The effects of TGF-β and EGF on ChAT activity were evident within 4 days of continuous exposure to this growth factor. However, although the dose-response profiles of TGF-β and EGF on ChAT enzymatic activity were similar (i.e. maximal inhibition of ChAT activity occurred with 10 ng/ml, TGF-β) consistently produced a more pronounced inhibition of ChAT activity when compared to EGF in situ cultures. When we compared the effects of TGF-β and EGF on ChAT activity, for those with EGF; the decrease in ChAT activity was not additive, suggesting a common mechanism of action for both factors. In addition, EGF induced a marked proliferation of astrocytes and microglial cells in the fetal septal cultures without affecting the numbers of ChAT-positive cells. The inclusion of the antibiotic 5-fluorodeoxyuridine completely abrogated both the glial cell proliferation and the decrease in ChAT enzymatic activity induced by TGF-β. Consequently, TGF-β is indirectly affecting cholinergic cell differentiation via glial cells. Competitive binding studies on different cell populations from the medial septum suggested that TGF-β is more effective in displacing EGF binding to its high affinity receptor, whereas, the opposite is seen for the low affinity EGF receptor. Finally, although TGF-β when applied in the absence or presence of an antagonist did not appear to affect the number or glutamic acid decarboxylase (GAD) immunoreactive neurons (GABAergic) as assessed by GAD immunocytochemistry, the intensity of the immunoreaction appeared to be somewhat higher in TGF-β treated cultures when compared to controls. Quantitative effects of TGF-β on GABAergic neuronal morphology were determined by morphometry. These results suggest that TGF-β can differentially affect neuronal and glial cell populations in the developing rat medial septal area both directly and indirectly.


TGF-β and TGF-β2 are pleiotropic cytokines with prominent roles in the regulation of cell proliferation, migration, differentiation, and extracellular matrix. We have previously found (Flinders et al., Development 113: 1991; Bieger et al, SAK 1999) that sympathomimetic neuronal populations (in the embryonic mouse adrenal gland as well as adult rat and bovine chromaffin cells) synthesize and release TGF-β. Western blotting using an antibody to the TGF-β receptor type II indicates that promulgation of bovine adrenal medulla to have a TGF-β receptor type II component. Chromaffin cells of the rat adrenal medulla are capable of divide in a decreasing fashion, from birth to adulthood (Tischler et al., Int. J. Dev. Neurosci, 7: 1989) and NGF, TGF-β and TGF-β2 are known to stimulate chromaffin cell proliferation in vitro (Brodin and Gammeltoft, PNAS 91: 1994). Since a physiological role for the chromaffin cell-derived TGF-βs has not been established as yet, we were interested whether TGF-β would interfere with the capacity of chromaffin cells to divide and respond to various mitogens. Chromaffin cells were dissociated from PS rat adrenal medullae as previously described and cultured for 7 days under serum-free conditions. Immunoreactivities of TGF-β, IGF-II, and IGF-I are expressed by single or in combinations. BrDU was added at 10 μM during the final two days. Cultures were then processed for the co-localization of BrdU- and tyrosine hydroxylase immunoreactivities. TGF-β, IGF-II and RA, applied single or in combinations, caused up to 50-fold increases in chromaffin cell proliferation. TGF-β, applied at 1, 2.5, or 10 ng/ml, significantly reduced the factor-mediated increases in proliferation. This suggests that TGF-β, released by chromaffin cells, together with glucocorticoids and PACAP (Brodin and Gammeltoft, 1994), which is released from intramedullary nerve terminals, must be considered as negative regulators of chromaffin cell division.


Insulin and insulin-like growth factors (IGF-I, IGF-II) are closely related polypeptides which are found in the CNS and are known to regulate neuronal survival and neurite outgrowth. They are each associated with specific cell surface receptors and several soluble binding proteins (IGBFs) which are involved in regulating function and availability. The primary analogues of IGF-I were insulin and IGF-II, insulin with basic analogues (5Glh, 5Ala, T1yr, 5Leu) of basic IGF-I and a B chain analogue in which the first 16 amino acids of IGF-I were replaced by the first 17 amino acids of insulin. These analogues have been significantly reduced binding affinity for IGF-I receptors. Neuronal cultures were established using embryonic (E15 - E17) rat cortex and PitD4 sarcoma cells in order to determine whether these IGF analogues were as effective as native human IGF-I as peptide to promote neuronal survival and neurite outgrowth. They are each associated with specific cell surface receptors and several soluble binding proteins. Cultures were maintained in DMEM+10% FCS in the presence of mitotic inhibitions for 3 days (cerebellum) and 6-8 days (cortex). DMEM was then replaced with Locke's solution containing no glucose. In some cases,洛克's Locks containing IGF-I or analogues. After 48 hours, Locke solution was removed and the remaining cells were assayed for LDH, an established method for assessing cell viability. These results are expressed as a percentage control and in the presence of either IGF-I or either of the IGF-I analogues the percentage LDH released from cortical and cerebellar neurones was significantly reduced (approx. 50%) indicating cell survival. More importantly, the data show that the IGF analogues with reduced affinity for IGF-IRs are an effective as IGF-I in promoting cell survival and their reduced affinity for IGF-IRs has no deleterious effect on their neuroprotective function.

TRANSFORMING GROWTH FACTOR (TGF)-β1 REGULATES RAT BRAIN TGF-β TYPE II RECEPTOR mRNA LEVELS. T. E. Morgan*, D. K. Sarkar†, Y. W. Y. Ta, and C. E. Fitz. Anand Gerontoloty Center, Department of Biological Sciences, University of California, San Francisco, CA 94110; †Department of VCAPP, Washington State University, Pullman, WA 99164-6520, ‡Salk Institute, San Diego, CA 92186-5800.


ATA is known to protect neurons from apoptotic cell death by inhibiting of calcium-activated endonucleases. With PC12 cells, a neuronal model cell line, ATA prevents cell death under serum free condition. Inhibition of endonucleases by ATA was not observed in isolated nuclei of PC12 cells. The western blot analysis of PC12 cell lysate with anti-phosphotyrosine antibodies revealed that protein phosphorylated at 100 μM ATA for 2 minutes induced phosphorylation of the tyrosine residues of some proteins. The major tyrosine-phosphorylations induced by ATA were not observed in PC12 cells treated by NGF or EGF. However, ATA, as well as NGF or EGF, induced the tyrosine-phosphorylations of PI3 kinase and SHC in PC12 cells. The induction of tyrosine-phosphorylation by ATA was not observed in NIH3T3 cells. Moreover, ATA did not protect NIH3T3 cells from cell death under serum free condition.

These results suggest that the neuroprotective action of ATA is mediated by the activation of the subsequent stimulation of the tyrosine-phosphorylation cascade.
690.21

Cultured PC12 cells possess receptors for VIP, NGF, or EGF and can be differentiated into a neuron-like cell type in vitro by the addition of an array of peptide growth factors, including nerve growth factor (NGF) and EGF. Addition of either NGF or EGF to PC12 cells elicits activation of cognate tyrosine kinase (TK) receptors resulting in rapid, enhanced tyrosine phosphorylation of a series of substrates that is similar for the two growth factors. While NGF promotes neuronal differentiation of PC12 cells, EGF invokes a mitogenic response. The neuroactive peptide, VIP, has been reported to promote a partial neuronal differentiation in neuroblastoma cells, an effect that is also increased in CAMP levels. The possibility that a combination of EGF-stimulated TK activity and the CAMP-stimulating effect of VIP could support an NGF-like neuronal differentiation response in PC12 cells was investigated.

Co-administration of EGF and VIP to PC12 cell cultures for up to 6 days results in robust neurite outgrowth. The neurites approximate, in length, those obtained by NGF treatment for comparable periods of time, but are less dense in their arborization. In the presence of 300 nM VIP, the neurite outgrowth response can be obtained with concentrations of EGF as low as 1 ng/ml. The neuronal morphologic differentiation induced by EGF + VIP is not inhibited by the NGF-selective inhibitor, K-252a. These results suggest that peptides such as VIP and EGF, which are partial, or ineffective neurotrophic agents may interact cooperatively eliciting a neuronal morphologic differentiation similar to that observed for the identified neurotrophin, NGF. One possible inference suggested by these data is that, CAMP-dependent protein kinase and tyrosine kinase activities are sufficient to support neurite outgrowth, a characteristic feature of neuronal differentiation.

690.23

Colony stimulating factor-1 (CSF-1) was originally identified as a specific growth factor for cells in the mononuclear phagocyte lineage, and subsequently was also found to be an important factor in maternal-fetal regulation. We find that CSF-1 is a growth factor in the nervous system as well. Nuclear acid studies demonstrate that CSF-1 mRNA is expressed in vivo in developing and adult mouse brain in various regions, including hippocampus, striatum, cerebellum and cortex. CSF-1 mRNA is found in whole brain samples as early as embryonic day 12.5 and is detectable through adulthood with some sexual variation. RT-PCR analysis shows splice variants of CSF-1 mRNA are expressed which encode soluble, and not membrane-bound, growth factor. Additionally, mRNA for the CSF-1 receptor is readily detectable in the developing and adult CNS. Regional studies correlate CSF-1 expression with CSF-1 mRNA expression. Treatment of primary cultured neurons from five brain regions with CSF-1 results in neurite outgrowth.

To define the role of CSF-1 in vivo, electrolythrophic techniques were employed in mice, which are homogamous for an inactivating CSF-1 mutation. These animals display abnormal visual and auditory evoked potentials, using both extracranial and intracranial recordings, indicative of abnormal cortical function. The expression of CSF-1 in vivo, the effects of CSF-1 on neurons, as well as the neurobiological deficits in vivo suggest that CSF-1 is an important factor in CNS development.

690.25

LA-N-2 is a cell line derived from a human peripheral neuroblastoma. It has a partially cholinergic phenotype and is a potential in vitro model of peripheral cholinergic neurons. It does not demonstrate CDP-choline-stimulated, Ca2+-dependent release of acetylcholine (ACh). The object of this study was to induce Ca2+-dependent release of ACh in LA-N-2 cells with various differentiation agents.

Cells were cultured for 14 days in 1 ml dibutyryl cAMP (dBcAMP), 0.25 mM leukemia inhibitory factor (LIF) or/and 3.8 mM nerve growth factor, or 10 nM retinoic acid (RA) for 9-14 days in F12/HEPES with 10% FBS. Cells were loaded with [3H]choline for 30 min at 37° and washed. The amounts of cellular and released (5 min, RT) labeled and unlabelled ACh and choline were determined by HPLC. None of the differentiation agents induced Ca2+-dependent release of [3H]ACh. Some of them, however, had marked effects on other aspects of cholinergic function. LIF increased [3H]choline uptake, [3H]ACh synthesis, and ACh content 250% and Ca2+-independent release of [3H]ACh 70% compared to untreated cells. RA increased [3H]choline uptake, [3H]ACh synthesis, and ACh content 30%. A Ca2+-independent and basal release of [3H]ACh 130%. dBcAMP increased [3H]choline uptake 20% and decreased ACh content 60%. LA-N-2 cells may be a good model for studying aspects of peripheral cholinergic function.

690.26

Vasopressin receptors are thought to respond to VIP, was shown to be a nitrogen for sympathetic neuroblasts (Pincus et al., Nature 341,564,1990), and for human neuroblastoma (all line-HNB), a tumor of the sympathetic nervous system (Wollman et al., Brain Res. 624,339,1993). Northern blot analysis revealed VIP mRNA neuroblastoma cells (cell-line-HNB) suggesting an autocrine growth factor role for VIP in neurogenesis and tumor propagation. Measurements of thymidine incorporation and cell numbers now showed that a potent VIP hybrid antagonist(1-34)-VIP, Gozes et al., Endocrinology, 125,2945,1989) inhibited cell division in this neuroblastoma cell line. The hybrid VIP antagonist also inhibited lung cancer growth (Gomez et al., Proc. Nat. Acad. Sci. USA 90, 4145, 1993), indicating a new therapeutic strategy against prevalent cancers including neuroblastoma, the most common solid malignany of children less than 5 years of age.
691.1

EFFECTS ON DEVELOPMENTAL AND ADULT NEUROCHEMISTRY OF CRONIC SERATAL TREATMENT WITH AN INHIBITOR OF ORNITHINE DECARBOXYLASE IN THE RAT. A. Contestabile, M. Sparrasani, V. Virgili, F. Facchinetti and R. Gianu (Sapienza University of Rome, Italy).

Neonatal rats were treated from postnatal day 0 to 24 with daily s.c. injections of the suicide ornithine decarboxylase inhibitor, DPH. In addition to some somatic effects, the treatment resulted in permanent decrease of brain weight which was mainly due to a 40% decrease of the cerebellum. When ornithine decarboxylase inhibition was only effective during the first phase of the treatment, the levels of putrescine and spermidine were decreased in the cerebellum and forebrain up to 3 days after the end of the treatment. Significant alterations of neuronal and glial chemical markers were measured in the cerebellum of treated rats once adults. Developmental treatment with DPH activates a mechanism able to keep low levels of putrescine and spermidine even in the presence of a progressive lack of ornithine decarboxylase inhibition. Furthermore, low developmental levels of the two polymines mainly arise from regions where substantial neurogenesis does occur during the treatment, such as the cerebellum.

691.3

MOON ABNORMALITIES INDUCED BY X-IRRADIATION AT BIRTH ARE DELAYED BY PERINATAL GMI TREATMENT.


Since GMI was reported to enhance neurite formation after damage of central neurons by different agents, we studied its effects on motor abnormalities and neurochemical changes in cerebellum (CE) induced by 5 Gy single dose of X-rays (X) applied to rats up to 72 h after birth. GMI 30 µg/g.were s.c. injected using different protocols: 1) 4 daily doses after X; 2) 1 dose 1 h before and other immediately after X; 3) 3 daily doses after X and 3 daily doses after X. Noradrenalin (NA) was assayed fluorometrically and motor function was quantified using an “ad-hoc” test.

At postnatal day (PN) 30, indicators of motor function in X-treated rats that received GMI, no matter the protocol used, returned to non-irradiated values. However, at PN 90 no differences in motor function were found between GMI-treated and un.injected exposed rats, except for protocol 1. None of the protocols modified the changes in NA levels induced by X.

Then, GMI (in particular, when repeatedly administered after X) may produce a long-term delay in the appearance of X-induced motor abnormalities, probably due to an action(s) on central pathways involved in motor output.

691.5

RECOMBINANT HUMAN GLIAL GROWTH FACTOR: TEMPORAL KINETICS AND SIGNAL TRANSDUCTION IN SCHWANN CELLS.


Glia growth factors (GGFs) are products of the neuron gene family that are expressed in the nervous system (Marchioni et al., Nature 262:312-318,1993). Recombinant human GGF2 (rGGF2) is a potent mitogen for Schwann cells, and the rGGF2 action on Schwann cells in vitro: the temporal kinetics of DNA synthesis and activation of signal transduction pathways. Purified rGGF2 stimulates DNA synthesis in both a dose and time dependent manner. Exposure to rGGF2 for various time periods before the addition of a-thymidine labeling media stimulates DNA synthesis maximum fold increase added at 24 hr, and at 25 min when added at 7 hr and 20 min when added at times less than 2 hr (to 5 min).

In addition, we have used compounds that inhibit either tyrosine kinases or protein kinase C (PKC) to determine if the signal for DNA synthesis is acting through these pathways. We show that induction of DNA synthesis by rGGF2 is inhibited by either staurosporine, a PKC inhibitor, or genistein, a tyrosine kinase inhibitor. Our data suggest that rGGF2 can stimulate DNA synthesis in Schwann cells through two different signal transduction pathways, PKC-mediated as well as a receptor-tyrosine kinase pathway. We also examined the early events stimulated by rGGF2 on second messenger pathways that signal directly to the nucleus. Within 1 hr of treatment, rGGF2 activates several transcription factors to bind to DNA. These data describe some of the earliest events that are initiated by the binding of GGF2 to the high-affinity cell surface receptor on Schwann cells that lead to the generation of a mitotic signal.

Because rGGF2 is an important growth regulator for several distinct cell types, our goal is to determine the biochemical basis for these pleiotropic responses induced by rGGF2 in different cell types and tissues.

691.6

A ROLE FOR SCHWANN CELL-ASSOCIATED HEPARAN SULFATE IN MODULATING THE ACTIVITY OF THE NEUREGULIN, rGGF2.


Glia growth factor 2 (GGF2) is a potent Schwann cell mitogen, which is expressed in neurons and produces several products of the neuregulin family (Marchioni et al., 1993, Nature 262:312-318). Previously Ratner and co-workers (PNAS 85:6992-6996, 1988) described a neuronal-associated Schwann cell mitogen bound to heparan sulfate proteoglycan (HSPG) whose mitogenic activity can be inhibited by heparin. Several products of the neuregulin family have been purified from several sources, including chromatography, and Schwann cells have HSPG associated with their plasma membrane and their basement membrane (Carey et al., 1987, JCB 108:1013-1025). We have found that heparin competes for the biological activity of rGGF2. We show that heparin inhibits the mitogenic activity of rGGF2 and blocks pituitary tyrosine kinase activation on Schwann cells. In contrast, 2 µM glucosamine, and 2 µM sodium sulfite, have no effect. Heparin added to other known Schwann cell mitogens (rhFGF, PDGF-ββ) produced little or no inhibition. When Schwann cells were treated with 4-methylumbelliferyl-β-D-xyloside to inhibit proteoglycan assembly, we observed a nearly total loss of mitogenic response to rGGF2. We hypothesize that rGGF2 interacts with Schwann cell membrane rGGF2 a way through cell-surface HS to mediate its effects. This co-receptor model of biological activity, which consists of HS and a high affinity tyrosine kinase receptor for maximal activity is well-supported for other heparin-binding growth factors (Rapaginas et al., 1991, Science 252:1705-1707; Higashiyama et al., 1993, JCB 122:939-949).
691.7

**PROMOTION OF PERIPHERAL NERVE REGENERATION BY A SOLUBLE NEUREGULIN, hRGGF2: STUDIES OF AN ENTUBULATION MODEL IN VITRO**

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Glial growth factors (GGFs) comprise a group of proteins, first identified in brain extracellular matrix and shown to promote the regeneration of Schwann cells in vitro (Marchioni et al., 1993, Nature 362:312-318). Recombinant human GGF2 (hRGGF2) is one of several differentially-spliced products of the neuregulin gene. We have previously shown that hRGGF2 promotes Schwann cell proliferation without significantly affecting the expression of a number of Schwann cell associated proteins (Bermingham-McDonogh et al. 1993, Soc. Neurosci. Abstr. 19:698). The proliferative activity of this molecule has been shown to be of therapeutic utility in peripheral nerve damage and disease. Since Schwann cells are known to provide trophic support for a variety of neural populations, simple expansion of the resident Schwann cell population in a site of disease may promote regeneration and survival.

The goal of the current study is to examine the therapeutic utility of GGF2 in the promotion of nerve regeneration by analyzing an in vitro model of the entubulation surgery commonly used to treat peripheral nerve gap injuries. In the current model, a fragment of neonatal rat superior cervical ganglion is placed in one end of a segment of polyethylene tubing (1.9 mm x 10mm) that has been filled with culture medium containing bovine dermal collagen ± hRGGF2. The collagen is allowed to gel and the tubes are maintained in culture for up to 10 days. The results suggest that hRGGF2 promotes Schwann cell proliferation, and Schwann cell invasion of the tube in a dose-dependent manner.

691.9

**GLIAL GROWTH FACTOR 2, A NOVEL MUSCLE TRACTION FACTOR**


Recently, the expression of one or more products of the neuregulin gene in peripheral nerve and motor neurons has been described by in situ hybridization (Marchionni et al., Nature 1993;362:312-318). One of these neuregulins, acetylcholine receptor-inducing activity (ARIA), has been shown to stimulate the expression of several genes encoding components of the developing neuromuscular junction in chick muscle culture (Falls et al., Cell 1993;72:601-615, Corfas & Flachsbart, J. Neuroscience 1993;13:2118-2125).

A secreted form of neuregulin, hRGGF2, has been cloned, based on its mitogenic activity on Schwann cells, and expressed in CHO cells. The possibility that hRGGF2 may have trophic effects on muscle cells in culture was investigated. hRGGF2 was mitogenic for subconfluent quiescent human myoblasts, but did not inhibit myoblast fusion. Differentiation of clonal human myoblasts was not affected in presence of hRGGF2 receptors. After 6 days of differentiation (Sklar, et al., J. Cell. Biochem. 1994;54:540), the increase in myoblast number was the result of an inhibition of myotube death as measured by propidium iodide uptake. hRGGF2 effects on muscle culture suggest that this neuronal-derived factor may regulate muscle growth in vivo.

691.11

**β-ACTININ IS REGULATED BY SYNAPTIC ACTIVITY AND IS EXPRESSED IN THE DEVELOPING RAT BRAIN**

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β-ACTININ is a member of the transforming growth factor-β family of superfamily of secreted proteins. By differential screening, we have identified it to be rapidly regulated by neuronal activity. β-ACTININ mRNA is induced in the dentate gyrus with a single monosynaptic input in a region where it is not expressed as measured by in situ hybridization (Marchionni et al., Nature 1993;362:312-318). A secreted form of neuregulin, hRGGF2, has been cloned, based on its mitogenic activity on Schwann cells, and expressed in CHO cells. The possibility that hRGGF2 may have trophic effects on muscle cells in culture was investigated. hRGGF2 was mitogenic for subconfluent quiescent human myoblasts, but did not inhibit myoblast fusion. Differentiation of clonal human myoblasts was not affected in presence of hRGGF2 receptors. After 6 days of differentiation (Sklar, et al., J. Cell. Biochem. 1994;54:540), the increase in myoblast number was the result of an inhibition of myotube death as measured by propidium iodide uptake. hRGGF2 effects on muscle culture suggest that this neuronal-derived factor may regulate muscle growth in vivo.

691.8

**CONTROLLED RELEASE OF hRGGF2 FROM A MICROPOROUS COLLAGEN/COMPOSITE MATRIX.**


To maximize the therapeutic potential of growth factors in nerve regeneration, it is desirable to control their delivery to the site of the lesion. Recombinant human glial growth factor 2 (hRGGF2), a product of the neuregulin gene, stimulates rat Schwann cell proliferation and DNA synthesis in cell culture (Marchionni et al., Nature 1993;362:312-318). The excessive biological activity is less than 24 hours when soluble hRGGF2 is added to a polyethylene nene guide tube reconstituting the myelin sheath of sciatic nerve. The initial goal of this study was to determine whether hRGGF2 can be immobilized and released in quantities sufficient to enhance Schwann cell proliferation within a nerve guide tube. This technology will then be employed in a rat sciatic nerve model of nerve repair. A composite microporous structure incorporated with hRGGF2 was designed for this purpose. hRGGF2 was exposed to this microporous membrane (thickness 150 μm) under mildly acidic conditions. Under these conditions secondary bonds within the collagen matrix are broken; and reform when pH is raised in a manner that incorporates hRGGF2 within the membrane.

Employing stimulation of Schwann cell [3H]-uridine incorporation in vitro as a marker for release of hRGGF2, the rate of release was determined to exceed 0.5 ± 0.1% per mm2 of membrane surface over a period of at least two weeks. A mathematical model of a 1:4 ratio of the nerve guide tube lined with this membrane predicts that at the aforementioned release rate, [3H]-hRGGF2 would increase at a rate of >30 pMolar per hour. Based on preliminary estimates of the release rate, the [3H]-hRGGF2 was incorporated into the tube in vivo in rats, and an EC50 of ~0.15 μM for stimulation of Schwann cell proliferation [3H]-uridine incorporation) in vivo, the model predicts that by this means of controlled release, [3H]-hRGGF2 will exceed that required for stimulation of Schwann cell mitogenesis for a two week period.

691.10

**NEUREGULIN. hRGGF2, STIMULATES TRANSLOCATION OF THE ACHR DELTA SUBUNIT GENE.**


Motor neurons induce the expression of activin A receptors in situ and in vivo. NEURO-2A neuroblastoma cells, which express activin receptors (ACHR) at neuromuscular synapses. We have shown that a signal in the synaptic basal lamina at the neuromuscular synapse stimulates translocation of the ACHR δ subunit gene in the myotube nuclei of skeletal muscle. (Marchionni et al., Nature 1993;362:312-318). A secreted form of neuregulin, hRGGF2, has been cloned, based on its mitogenic activity on Schwann cells, and expressed in CHO cells. The possibility that hRGGF2 may have trophic effects on muscle cells in culture was investigated. hRGGF2 was mitogenic for subconfluent quiescent human myoblasts, but did not inhibit myoblast fusion. Differentiation of clonal human myoblasts was not affected in presence of hRGGF2 receptors. After 6 days of differentiation (Sklar, et al., J. Cell. Biochem. 1994;54:540), the increase in myoblast number was the result of an inhibition of myotube death as measured by propidium iodide uptake. hRGGF2 effects on muscle culture suggest that this neuronal-derived factor may regulate muscle growth in vivo.

691.12

**TRANSMISSION ELECTRON MICROSCOPY REVEALS A REDISTRIBUTION OF MAP2 IN NEURO-2A NEUROBLASTOMA CELLS AFTERS G A N I T R O S I D E TREATMENT.**


Our previous immunofluorescent studies of Neuro-2A cells exposed to ganglioside GM1 demonstrated enhanced microtubular (MT)-dependent, increased microtubular network density, and a redistribution of MAP2 from the perikarya to the distal neuritic processes. In the current study, immunogold-labeled specimens were analyzed with transmission electron microscopy. Neuro-2A neuroblastosoma were grown in vitro with or without GM1 (150 μg/ml) for 24 hr and embedded in Lowycryl. Sections were incubated with anti-tubulin and anti-AMAP2 and colloidal gold labeled secondary antibody, and the number of gold particles per μm2 was determined in the microtubules, neuritic processes and the perikarya of a minimum of 50 cells per condition. The results demonstrated that: (a) GM1 increases the area of spine-like projections (p<0.05); (b) more MAP2 per unit area was found in spine-like projections after GM1 treatment (p<0.001); (c) more MAP2 was exposure (p<0.02); (d) MAP2 seems more closely associated with actin-rich subsynaptic cytoplasm than with MT. In contrast, the location of tau protein, another MAP, was not changed after GM1 treatment. These observations are consistent with previous qualitative studies demonstrating that GM1 increases the spine-like projections. Since MAP2 is associated with actin in dendritic spines, perhaps GM1 shifts MAP2 from interactions with MT to a more direct interaction with cytoskeletal filaments. This study is in progress to determine the effect of GM1 on the synthesis of MAP2 and tau protein. Supported by a grant from Alliant Community Trust Fund, Louisville, KY.

A role of thrombin in neuropathological processes is suggested by the fact that this protein is secreted by glial cells. An imbalance between thrombin and FN-1 (its natural inhibitor) seems to be involved in the pathogenesis of human neurological diseases including Alzheimer’s disease. In vitro thrombin alters neurite outgrowth and, at low concentrations, increases choline acetyl transferase (ChAT) activity in a mixed neonatal myocerebral culture of septal cells. To characterize further the effect of thrombin on cholinergic neurons we have compared the effect of thrombin and of the 14-amino acid peptide agonist of the thrombin receptor on a pure culture of septal neurons and on co-cultures of septal neurons/glial. Septal cells were grown in defined medium, onto a monolayer of glial cells for co-cultures. Cells were treated one day after plating. ChAT activity and MT1 reduction (index of cell viability) were assayed at the 5th day in vitro.

In pure septal neurons, low concentrations of thrombin (up to 30 nM) did not affect ChAT activity or MT1 reduction. However, 100 nM thrombin decreases ChAT activity and MT1 reduction by 44% and 17%, respectively. In co-cultures (17% at 0.1 nM and -63% at 100 nM) and in co-cultures (-4% at 0.1 nM and -28% at 100 nM). This peptide did not affect MT1 reduction. Thus, thrombin’s effects on cholinergic neurons seem to be mediated, at least in part, by thrombin receptors and glial cells seem to play a major role in thrombin action.
CHARACTERIZATION OF HCNP PROCESSING ENZYME

HCNP is a peptide which stimulates acetylcholine synthesis in medial septal nuclei explant culture. The peptide consists of eleven amino acids located at N-terminal lesion of its pro-protein, and suggested to be processed by cleaving enzyme. In the present study, we characterized the HCNP processing enzyme in Wistar rat hippocampus. Hippocampus from 10-11 day old rats were sonicated in 50mM MES-NaOH buffer (pH6.0) containing 150mM NaCl, 3mM KC1, 1mM EDTA and 1mM DTT, and centrifuged at 15000rpm for 20min. The supernatant was incubated at 37°C with purified HCNP pro-protein or synthetic peptide which consisted of eleven amino acids from N-terminal of the HCNP pro-protein. The reaction was terminated by adding TFA and subjected to HPLC equipped with C18 column to measure the enzyme activity. The enzyme was eluted between 58-10%Ac in the molecular sieving column, and retained on DEAE column at pH7.6 and G-Butyl column at pH6.0. Optimal pH of the enzyme activity was 5.6, and the activity was inhibited by Chymostatin and E-64.

NUTRITIONAL AND PRENATAL FACTORS


Prematurely-born infants have higher heart rates and reduced heart rate variability at term relative to fullterm neonates (Eiselt et al., 1993), suggesting that prematurity may have long-lasting effects on cardiovascular control. In the present study, we assessed post-term development of heart rate and its variability in prematurely-born infants who continue to suffer from apnea of prematurity (AOP).

Six-hour recordings of EKG and respiration were obtained from infants with AOP born at gestational ages of 24 to 35 weeks. Heart rate and its variability were assessed during three periods of regular respiration in each recording of the premature infants and similar recordings of fullterm infants as controls. At term, infants suffering from AOP showed higher heart rates and reduced heart rate variability relative to fullterm neonates. Over the first month of postfullterm infancy, however, the premature infants showed an increase in heart rate and a reduction in variability, such that by 1 mo after term, there were no significant heart rate differences between fullterm infants and prematurely-born infants suffering from AOP. The two groups of infants continued to show comparable heart rates and variabilities at all ages up to 6 mo after term. Thus, neither prematurity nor AOP appears to have a long-lasting effect on cardiac rate or overall cardiac variability.

SUPPORTED BY HD-22895.


Restrain stress applied to pregnant rats during late pregnancy results in offspring exhibiting some behavioral and neurochemical alterations. Particularly, this procedure of prenatal stress can cause long-lasting changes in the hypothalamo-pituitary-adrenal axis of the offspring, that persist in adult animals. Moreover, prenatally stressed rats show an increase in heart rate and a reduction in variability, such that by 1 mo after term, there were no significant heart rate differences between fullterm infants and prematurely-born infants suffering from AOP. The two groups of infants continued to show comparable heart rates and variabilities at all ages up to 6 mo after term. Thus, neither prematurity nor AOP appears to have a long-lasting effect on cardiac rate or overall cardiac variability.

SUPPORTED BY HD-22895.


The effects of gestational protein malnutrition on the establishment, maintenance, and function of the GABAergic neuronal system and related behavioral alterations have been investigated in young adult rats. Prenatal malnutrition was induced by feeding rats a diet containing 20% casein, 20% bran, 20% sucrose, 20% corn starch, and 10% lard. The rats were weaned to a chow diet at weaning and fed this diet throughout the experimental period. The effects of prenatal malnutrition on the development of the GABAergic neuronal system were studied using a variety of techniques, including autoradiography, electron microscopy, and behavioral testing.

RESULTS: (1) Prenatal malnutrition results in a decrease in the number of GABAergic neurons in the cerebral cortex of young adult rats. (2) The GABAergic neuronal density is decreased in the cerebral cortex of young adult rats. (3) The GABAergic neuronal density is decreased in the cerebral cortex of young adult rats. (4) The GABAergic neuronal density is decreased in the cerebral cortex of young adult rats. (5) The GABAergic neuronal density is decreased in the cerebral cortex of young adult rats.


The effects of prenatal protein malnutrition on the establishment, maintenance, and function of the GABAergic neuronal system and related behavioral alterations have been investigated in young adult rats. Prenatal malnutrition was induced by feeding rats a diet containing 20% casein, 20% bran, 20% sucrose, 20% corn starch, and 10% lard. The rats were weaned to a chow diet at weaning and fed this diet throughout the experimental period. The effects of prenatal malnutrition on the development of the GABAergic neuronal system were studied using a variety of techniques, including autoradiography, electron microscopy, and behavioral testing.

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The effects of prenatal protein malnutrition on the establishment, maintenance, and function of the GABAergic neuronal system and related behavioral alterations have been investigated in young adult rats. Prenatal malnutrition was induced by feeding rats a diet containing 20% casein, 20% bran, 20% sucrose, 20% corn starch, and 10% lard. The rats were weaned to a chow diet at weaning and fed this diet throughout the experimental period. The effects of prenatal malnutrition on the development of the GABAergic neuronal system were studied using a variety of techniques, including autoradiography, electron microscopy, and behavioral testing.

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693.5
OXIDATIVE STRESS RISKS WITH HIGH POLYSATURATED FATTY ACID DIETS IN BRAIN/HEART/KIDNEY/MAMMARY/LIVER OF FEMALE SHR RATS
R.S. Mehta, C.A. Gunnett and D.K. Hartle. Dept of Pharmaco and Toxicol., College of Pharmacy, Univ. of Georgia, Athens, GA 30602.

Aging can be exacerbated by increasing the rate of initiation of random free radical reactions or by increasing ingestion of easily oxidized dietary components. Dietary polysaturated fatty acids (PUFA's) of omega-3 and omega-6 type are known to have some antioxidant vascular effects that are susceptible to peroxidation under multiple conditions that encourage oxidative free radical formation. We studied the effects of long-term feeding with dietary omega-3 and omega-6 PUFAs on 1) blood pressures and 2) in vivo tissue lipid peroxidation in female spontaneously hypertensive rats. After 16 weeks of feeding with either control (5% corn oil, AIN) or high fat diet (19% Menhaden oil, 4% corn oil, 28% P UFA's), no differences in blood pressure were observed among groups. In whole brain homogenates, autoxidation averaged 282% and iron-ascorbate catalyzed peroxidation 1225% compared to baseline oxidation in all diet groups. Marked differences occurred between the omega-3 and omega-6 (MO and CO respectively) high P UFA diets on baseline oxidation, autoxidation and iron-ascorbate catalyzed oxidation in various tissues. MO caused a 67.5% increase in basal oxidation. 2624% increase in autoxidation and 4244% increase in iron-ascorbat catalyzed oxidation in mammary gland compared to the CO diet. Similarly, a 400% increase in iron-ascorbate catalyzed oxidation occurred in liver, 225% in heart, 120% in kidney and 50% in aorta in MO compared to CO, respectively. The results indicate that 1) brain is extremely susceptible to iron-catalyzed peroxidation; 2) neither high omega-3 nor omega-6 PUFA diet protects against peroxidation in female SHRs; and 3) high omega-3 fatty acid diets markedly increase the oxidative aging risk of multiple organs.

693.6

There has recently been increased research in studying the association between chemical imbalances and violent behavior. In the treatment of over 30000 assaultive young males, The Carl E. Figeller Treatment Center has observed elevated blood copper:zinc ratios. The objective of this study was to determine if violent and non-violent patients had significantly different copper and zinc levels and copper:zinc ratios. The sample consisted of all male subjects of the Center and for an 8 week period that were 3 to 18 years old. These subjects had a history of frequent assaultive incidents, while the control subjects had an absence of such incidents. There were no significant differences in copper:zinc ratios of 1.40 for violent and 1.02 for non-violent were shown to be significantly different (p < 0.05). The mean serum copper levels were 98.5 and 86.6 mcg/dl and plasma zinc levels were 75.4 and 87.4 mcg/dl for violent and non-violent subjects, respectively.

These relationships suggest a strong correlation between assaultive behavior and abnormal metal metabolism. Clinical studies are underway to determine if correcting these chemical imbalances will result in improved behavior.

693.7
EFFECTS OF PROTEIN DEFICIENT DIETARY REGIMENS, PRE-TERM BIRTH AND HYPERTENSION ON RAT NEONATAL BEHAVIOR. F. Diagros, E. Di Leo, M. F. Di Grav. , L. Le Presti*. Institute of Pharmacology, University of Catania Medical School, 95125 Catania, Italy.

The effect of various metabolic factors on the development of neonatal reflexes and on learning and memory capacity in adulthood was studied in the rat. Female rats of the Wistar strain were assigned to one of seven diets: a control regimen (commercial animal food at a reduced weight regimen of 80% diet containing casein as the only protein component). Another group of animals was subjected to a caesarean cut at day 19 of pregnancy in order to induce a pre-term birth of pups. Spontaneous hypertensive rats (SHR) were used to examine the possible influence of gestational hypertension on brain maturation. We found that protein deficient dietary regimens were accompanied by a retardation in the development of neonatal reflexes and a reduction in learning and memory capacity in adulthood. Differences were also found between control animals and pups born by caesarian cut or from SHR mothers, above all for eye opening time and body weight.

693.9
REPRODUCTIVE SUCCESS IS COMPROMISED IN RATS OVERTREATED AS NEONATES. L. Taylor* and L. Diaz. Dept. of Psychology, University of Washington, Seattle, WA 98195.

On postnatal day 4, Long-Evans female rat pups were randomly assigned to one of three groups: 1) mother reared in litters of 9 (MR), 2) gastrostomy-fed for ten days to match the growth of the MR group (WM), and 3) gastrostomy-fed with excess formula to accelerate growth (OF). On day 14 the gastrostomy-fed animals were returned to lactating dams in litters of 8 pups each. All animals were weaned on day 22 and maintained thereafter on laboratory chow. Plasma glucose readings were taken using tail blood. The animals were bred the following day. On day 190 glucose readings were again recorded. 6.3% of the 67 animals (67%) of 11 WM females (45%), and 4 of 11 OF females (36%) produced litters. Pregnant glucose levels of the successful females were significantly lower than glucose levels of females who failed to produce pups (p < 0.01). Pre-pregnant glucose levels were significantly higher in OF females and an analysis of variance also indicated that pre-pregnant glucose levels were different among the three groups (p < 0.01, ANOVA; OF x WM, p < 0.05, t test; OF x MR, p < 0.01, t test).

These findings support the hypothesis of several researchers that high maternal gestational weight gain can permanently alter metabolism. The issue of whether or not central regulatory control systems were altered in addition to peripheral metabolic systems by these nutritional manipulations in early development remains to be determined.

693.10

The effects of postnatal malnutrition (9% casein diet) in the somatic size, length of the apical dendritic, number of dendritic branching and the thorny excrecence area of CA3 hippocampal pyramidal cells were studied in rats of two ages (30 and 90 days old). A total of 144 rats imaged with the morphometric software were used for morphometric analysis. Results showed that postnatal malnutrition produced significant decreases (p < 0.05) in all the parameters studied. These findings indicate that postnatal malnutrition produces severe alterations in the morphatical and development patterns which in these cells occurs during postnatal life. We have found similar results in pre and postnatal malnutrition studies (Garcia-Ruiz M et al., Brain Res. 625 202:1993). Thus, only malnutrition can affect the functional integrity of the hippocampal activity of the CA3 field. Supported by DGAPA IN-204892, IN-204093 and CONACYT fellowship 83601.
692.11 PRENATAL MALNUTRITION ON THE BASKET (GABAergic) DENTATE GRAY CELLS IN THE RAT. A. Aguilar†, S. Diaz-Cintia, A. Gonzalez, M. A. Morales†, T. Kemperer and P. J. Goodlett. Centro de Neurobiologia, UNAM, UISS-IMP, SSA, c.p.14110, IBM, Mexico, D.F. 04510 and Boston Univ. School of Med. Center of Behav. Dev. and Retardation. M921, 80 East Concord Street Boston, MA 02118.

The effects of prenatal malnutrition (6% casein diet) on the somatic size, in basket cells was measured in the fascia dentata in rats of 30 days of age, using the Golgi technique. The density of GABA-like immunoreactive cell was studied in the fascia dentata and hippocampal formation, divided into three levels (rostral, medial and caudal). Results showed a significant reduction in the major axis (57%) and perimeter (18%) and 20% in uniform cells. In all three levels, numbers of GAD-immunoreactive cells were significantly increased in the hilus of fascia dentata in relation with the hippocampus. The bigger amounts of those cells were located in the middle part of the fascia dentata. In addition, preliminary data showed increased number of these cells in an older age (i.e. 220 days). The morphological alterations observed in these cells may affect at least in part, the functional integrity of the hippocampal formation, since they have inhibitory activity on the granule cells. Supported by DGAPA IN-204892 and IN-204093.  

692.13 THE SEVERITY OF NEONATAL ALCOHOL-INDUCED CEREBELLAR PURKINJE CELL LOSS IN RATS DEPENDS ON THE TIMING OF ALCOHOL EXPOSURE: A STEREOELECTOMIC STUDY. J. D. Thomas†*; C. B. Goodlett†, R. A. Wissmeier, R. McCleary and A. Aguilar. Alcohol Research Group of Iowa, Iowa City, IA 52242; University of Iowa, Iowa City, IA 52242.  

Short term binge-like alcohol exposure during the brain growth spurt in rats depletes Purkinje cells in the cerebellum, but the severity of loss depends on the timing of exposure. Two consecutive days of binge-like alcohol exposure beginning before or after, postnatal day (PD) 7 significantly reduces the density of Purkinje cell profiles in the cerebellar vermis (Bennet & Wissmeier, Clin. Exp. Neurosci., 1993). These findings, however, were based on biased counting methods from which estimates of total Purkinje cell number could not be obtained. The present study investigated the effects of two-day alcohol exposure on Purkinje cell number using a stereomicroscopic method. The optical fractionator technique which provides an unbiased estimate of total cell number. Sprague-Dawley rat pups were randomly assigned within litter to five treatment groups. Three alcohol-exposed groups were characterized and artificially reared from PD 4 through 9. All alcohol-exposed groups received 6.6 g/kg/day of alcohol, producing peak blood alcohol concentrations of 190 mg/dl. One group was exposed to alcohol on PD 4 & 5 (4/5), a second group on PD 8 & 9 (8/9), whereas a third group was exposed during both periods (Comb). Control groups included the same rearing condition and were not reared. On PD 55, subjects were perfused, the cerebellum was sectioned, and Purkinje cell number was determined with an Olympus/BICO computerized stereological system. Purkinje cell numbers were reduced in all alcohol-exposed groups (Comb: 53% of GC; PD 4/5: 57% of GC; PD 8/9: 83% of GC). The PD 4/5 group did not significantly differ from the Comb group as there had significantly more severe cell loss than the PD 8/9 group. These results confirm the greater susceptibility to alcohol-induced Purkinje cell loss early in the neonatal brain growth spurt. Moreover, these unbiased methods now show that the severity of cell loss is greater than previously estimated and that even exposure late in the brain growth spurt can produce significant reductions in Purkinje cell number. Supported by Grants AA05523 (JRW) & AA05996 (CRG).  

692.15 INCREASED LATE DURATION IN PAIRED COMPARISONS BY RHEUS MONKEY INFANTS WITH N-3 FATTY ACID DEFICIENCY (N-3 FAD). A. Retzbach*, N. Neuhring and E. Gohl. Oregon Health Sci. Univ., Portland, OR 97201 and Oregon Reg. Primate Res. Center, Beaverton OR 97006  

N-3 fatty acids are integral components of neural and retinal membranes. Peripherally deficient primates and human infants show low levels of n-3 fatty acids in cerebral cortex and retina, delayed development of visual acuity and abnormal electroretinograms. Nine infants deficient both pre- and postnatally and 8 standard nursery infants were derived paired comparison tests at 2, 5, and 13 weeks of age. Each test was given both 2 pattern-pairs and 6 face-pairs, each set presented on a separate day. Familiarization consisted of 30 sec of fixation. Tests (familiarized vs novel card) were 10 sec on each side, semi-randomized. For each stimulus set, tests were conducted both immediately and after 24-hours.  

Fixations by deficient infants were consistently longer in both the immediate and 24hr tests for patterns (p<0.05) and approached significance for faces (p<0.08). Table 1 shows the data for the Pattern Set (6 pairs) Face Set (6 pairs) Immediate 24-Hour Immediate 24-Hour Standard 1.18 +0.05 1.18 +0.06 1.20 +0.05 1.1 +0.05  

Deficient 1.52 +0.05 1.43 +0.06 1.48 +0.05 1.4 +0.05  

Look duration has been inversely associated with speed of visual processing. N-3 FAD may slow processing.  


The effects of postnatal malnutrition (6% casein diet) in the somatic size, number of dendritic branching and the spine density of granule cells were studied in rats of 30 day old. A total of 115 cells impregnated with rapid-Golgi were selected for morphometric analysis. Results showed that postnatal malnutrition produced significant decreases (p<0.05) in the number of spines in proximal and middle dendritic segments as well as dendritic density in the 7 of the 8 intercerebral rings, studied. These findings are similar to those found in the study of CA3 pyramidal cells and indicate that postnatal malnutrition produces severe alterations in the maturation and development patterns which in these cells occurs during postnatal life. Thus, these anatomical deficits may affect the functional integrity of the hipppocampal activity initiated in these granule cells. Supported by DGAPA IN-204892, and IN-204093, 2r-20891.  


It has been demonstrated that sensitivity to opiates declines as a function of multiparity in the rat. The impact of this maternal change on offspring sensitivity has not been studied. In order to determine if parity has an impact on offspring responsiveness to opiates, female Sprague-Dawley offspring from a first and second breeding were tested for their analgesic responsiveness to morphine. At 30-36 days of age subjects were tested for their hotplate latency 30 and 60 min after administration of 5mg/kg morphine. Prior to drug administration baseline latency was established. There was a significant effect (p<0.01) of parity on baseline and 30 min latencies, due to offset from the second mating showing higher latencies. The significance at 30 min was still present after correcting for baseline differences. It is not clear whether the observed difference is a result of maternal age (dams were 3 months old at first breeding and seven at the second) or parity. The data do, however, indicate that maternal history may significantly alter offspring sensitivity to drugs and possibly other aspects of behavior. (Supported by NIH-HD07228, UCLA Psychoneuroimmunology Program, and VA Medical Research Service.)  


Cerebral activity of normal (N) and malnourished (M) lactating female Sprague-Dawley rats was registered during a 24h period, during and after suckling and without their pups. Rats were malnourished 5 weeks before mating, throughout gestation and lactation. N rats showed a synchronized pattern during milk ejection. Using power spectra analysis in N rats, we found a significant increase of theta activity in the first third of the lactation period, an increase of delta waves in the second third and a significant increase of theta activity in the last third of this period. M rats showed a significant increase of theta activity during the last lactating days, however during the first third of the period they showed increase in delta waves. In both groups N and M delta waves increased when pups were retired from the nest. We also found ultradian rhythms in delta, theta and high frequency activity. N rats showed 4 main peaks and M rats presented 8 peaks, with phase advance in respect to N rats. These results suggest that protein malnutrition installed 5 weeks prior to conception, can produce alterations in the electrical activity of lactating rats (Supported by DGAPA IN-202891).
SLEEP-WAKE PATTERNS IN MALNOURISHED, REHABILITATED AND CONTROL RATS OF 60 DAYS OF AGE
A. Galvan*, L. Citrino and A. Alfaro Centro de Neurobiologia, UNAM, Mexico D.F. 04510.

This study was designed to evaluate the sleep-waking cycle of 6% protein malnourished, postnatally rehabilitated and control male Sprague-Dawley rats at 60 days of age. Malnutrition was established prenatally and was continued throughout gestation and postnatal life. Rats were rehabilitated by the "cross fostering" method switching at birth malnourished pups to well nourished dams. Occipital EEG and neck muscles EMG activity were obtained with bipolar stainless steel electrodes. Polysomnographic vigilance states were obtained for a 24 hour period and scored visually in 12 second epochs. Postnatally rehabilitated rats showed a similar distribution of their vigilance states as their controls during the light and dark phase, as well as in the 24 hour period. In contrast, malnourished rats showed an increased proportion of SWs during the dark phase and in the total 24 hour and a phase shift in the distribution of REM-sleep. These data reveal that malnutrition affects the mechanisms that regulate sleep-wake patterns and suggest that postnatal nutritional rehabilitation can prevent these alterations. (Supported by DGAPA IN-202891 and PADEP 30383).

EFFECTS OF CHRONIC UNDERNUTRITION ON THE AMPLITUDE AND LATENCY OF THE N13 AND N20 COMPONENTS OF THE SEP IN CHILDREN

An estimated 50% of Honduran children suffer from chronic undernutrition. To assess the consequences of such deprivation on CNS physiology, we measured the central conduction time (CT), using the somatosensory evoked potentials (SEP) in 14 children ages 7-10 with heights below the 3rd percentile for their age and 17 age-matched controls. The two groups also differed significantly in nutritional intake, socioeconomic variables, achievement in Bender's neuromotor test and hematocrit (control mean = 39; undern. = 36; p < 0.01, Scheffe F-test), but not birth weights. The children were tested at the median nerve with 0.3Hs shocks at a rate of 2-3Hz and a voltage sufficient to produce a just visible abduction of the thumb (motor threshold, MT). The cervical N13 and cortical N20 components were recorded over positions C5-C6 and C3-A1, respectively. In addition to motor threshold, recordings were also taken at 75% and 125%MT to assess the response to varying stimulation intensities. Mean CT (N20 latency-N13 latency) for the undernourished group (6.64ms) did not differ significantly from the controls (6.38ms, p=0.3), nor was there a difference in the amplitudes of the N13 and N20 waves with intensity. However, in N13 undernourished subjects whose hematocrit was extremely low the CT was 1.8 standard deviations greater than controls (p<0.001, t-test). Thus, CT may be affected in a proportion of subjects by a postnatal dietary lack, while the CNS of others may be "protected".

PHYSICAL ACTIVITY (PA) AND NEURAL ACTIVITY (NA) OF THE SOMATOSENSORY CORTEX IN MALNOURISHED AND CONTROL RATS
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We studied the response of the somatosensory cortex to stimulation of the contralateral hind paw in young malnourished and control rats. The somatosensory cortex was stimulated at 0.25 ms in the postnatal period and the PA and NA were recorded at the level of the rat's thalamus and sensorimotor cortex, respectively. The PA was increased in the control group compared to the malnourished group. The NA were recorded from the thalamus and the cortex. The NA were decreased in the malnourished group compared to the control group. These results suggest that the somatosensory cortex is undernourished due to a lack of nutritional intake.

In our previous studies of transplants of nigral tissue into dopamine (DA)-depleted rats, we found that the inclusion of embryonic striatal tissue increased the survival of these transplants. The territory that this was the greatest with the youngest striatal tissue containing primarily patch neurons (Costantini et al., Exp. Neurol. 1994). We have now employed dissociated cultures to examine whether the survival of DA neurons, as well as striatal patch neurons, is increased in co-cultures of nigral and striatal cells taken from different age embryos. Striatal patch neurons were labelled by in vivo BrdU injection on E13 + E14. The number of BrdU-labelled neurons was much higher in E14 striatal cultures (98%) as compared to E18 (10%) or E20 (4%) striatal cultures at 1 DIV, and the percentage in all cultures decreased over the next two weeks. The inclusion of E14 nigral cells attenuated this decline. Similarly, the number of DA (TH-immunoreactive) neurons in E14 nigral cultures decreased with time in vitro (10% at 1 DIV to 3% at 15 DIV), and this decline was attenuated by the inclusion of E14, but not E18 or E20, striatal cells. Thus, the survival of nigral DA neurons and striatal patch neurons in cultures appear to be enhanced by the presence of the other. These reciprocal influences may be relevant to the in vivo development of the nigrostriatal system, as well as the possible potentialization of transplanted cells. (Supported by MH46577.)

693.5 TRANSIENT c-fos EXPRESSION IN RESPONSE TO DOPAMINE D1 AGONISTS IN THE DEVELOPING STRIATUM. E. Arnaud, J. Arnaud, R.M. Bluhse*, J. Demotes-Mainard. INSERM U-394, 30077 Bordeaux, France.

In intact adult mice, dopamine D1 receptor activation only induces c-fos in the most caudal region of the striatum. In contrast, when dopaminergic receptors are prematurely sensitized by lesion of the nigro-striatal pathway, the expression of c-fos in response to D1 agonists is present throughout the striatum. Therefore, c-fos induction by D1 agonists requires a state of altered responsiveness of dopaminergic receptors. Since the D1 receptor develops in the striatum prior to the D2 subtype, we further examined in intact animals the postsynaptic ontogeny of the c-fos response to D1 agonists. D1 dopamine receptors are already present in the striatum at E14. Injection of the D1 agonist SKF 38393 results in a c-fos expression scattered throughout the striatum and observing a patchy distribution. After postnatal day 15 the D1 receptor-mediated c-fos hybridization signal had almost disappeared and lost its patchy expression, except in the most caudal division of the striatum where a strong expression appeared at postnatal day 3 and persisted in adults. Since this transient response of c-fos gene to D1 dopamine agonist parallels the developmental pattern of striatal dopamine innervation, of D1 dopamine receptor distribution and the delineation of the strionigral compartment, this study raises the questions of: i) the possible role of D1 receptor-mediated gene expression in the ontogeny of striatal compartments; and ii) the significance of a persisting D1-induced c-fos expression in the most caudal part of the adult caudate-putamen.

693.8 STAINING FOR ACETYLCOLINERESTASE REVEALS DIFFERENT PATTERNS IN THE THALAMUS AND CORTEX OF PERINATAL RATS, MICE, AND HAMSTERS. B. Shendure*, R. Chilale, and G.A. Pasantes-Maile. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699.

Acetylcholineesterase (AChE) is a marker for thalamocortical axonal terminals in perinatal rats and has been useful extensively to study the development of these fibers. Studies in which we attempted to use this marker in developing mice and hamsters have been striking differences in the expression of this enzyme in the three species. In developing rats, AChE staining heavily labels neurons in the ventral posterolateral (VPL) and ventral postero-medial (VPM) thalamic nuclei and reveals a somatotopically organized pattern of clusters in the developing cortex. In both hamster and mouse, VPM neurons were relatively lightly labeled by AChE. Staining in VPM appeared to be contained in afferent fibers. This was confirmed by its disappearance after partial lesioning of the spino-thalamic tract. Staining of both of these species resulted in a negative image of the distribution of thalamocortical fibers. This image appeared to result from reduced AChE cortical neuronal staining and first apparent as a crude representation of the body in laminas V and VI and then as a more precise image in layer II. In both AChE and Mlsl substance in developing mice indicated that the dense AChE staining was located in the septum. These results demonstrate a dramatic species difference in AChE expression by thalamic and cortical neurons and prompt caution in using AChE as a marker for developing thalamocortical axons. Supported by 5R 28688, DE 07734, and EY 08661.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
INFRAORBITAL NERVE TRANSECTION AND AXOPLASMIC TRANSPORT BLOCKADE HAVE SIMILAR EFFECTS UPON PRIMARY AFFERENT AND CENTRAL VIBRISSE-RELATED PATTERN FORMATION, THE TRIGEMINAL MAINSTEM COMPLEX. C.A. Bennett-Clarke, N.L. Chiala, R.W. Rhodes. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699

TRANSECTION of the infraorbital nerve (ION, the trigeminal branch that supplies the mystacial vibrissae) between P-0 and P-6 by application of implants impregnated with varying amounts of cholchicine or vinblastine, and, to a lesser extent, with a second-order neuron in the V brainstem complex. Such lesions cause a marked up-regulation of growth in the brainstem of damaged primary afferent axons and reductions in the staining of second-order cells for both cytochrome oxidase (CO) and parvalbumin (PV). Blockade of axoplasmic transport has less severe effects on the postnatal development of ION than transection and it does not disrupt peripheral activation in these cells. Nevertheless, such blockade produces changes in V primary afferent and second-order neuronal morphology that closely mimic those observed after nerve transection. Transport blockade causes a marked up-regulation in galvin V primary afferents and, in V nucleus principalis, V nucleus interopolaris, and the magnocellular part of V nucleus caudalis, the axons are arrayed in a clustered and somatotopic fashion which matches that of the mystacial vibrissae follicles. Transport blockade also causes reductions in the density of both CO and PV staining in the brainstem and a loss of the normally observed pattern indicating that correspondences neurology.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
694.1 EFFECTS OF IMPACTIONAL NERVE TRANSSECTION ON THE ORGANIZATION IN INTRAOPERATIVE CONNECTIONS WITHIN THE RAT'S PRIMARY SOMATOSENSORY CORTEX. P. A. King, A. R. Conover, C. A. Bennett-Clarke, N. L. Chiba, H. P. Killackey, and R. W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699

Intraoperative projections within layer IV of the vibrissa representation of the rat's primary somatosensory cortex (the barreled field) are generally restricted to adjacent layers and the dense aggregations of lamina IV neurons referred to as the barrels. In spite of the gross, anterograde and retrograde tracing with biotinylated dextran amine (BDA) we observed here, we failed to alter the organization of the intracortical connections in layer IV of the barreled field. Both retrogradely labelled cells and anterogradely labelled axons were present within the dense aggregations of granule neurons that remained in this lamina. In contrast, the pattern of intracortical connections in layer IV of rats with 10Hx transections on P-7 appeared normal. Labelled cells and anterogradely labelled axons was largely restricted to the septum and formed a negative image of the clusters of granule cells. These results suggest that altered input to the barrel cortex must be achieved very early in postnatal development to alter intracortical connections in this lamina.

Supported by DE 08971, DE 07734, and NS 28888


Mechanisms subserving pattern formation in the rat whisker-barrel system are unknown, although morphogenetic processes are necessary in part because NGF can preserve excess ganglion cells and alter pattern formation. Henderson et al. (1994) suggest that pattern form as a result of a neuroepithelial migration of enter neural cells that project to inter-whisker surfaces. If such cells project centrally to regions intervening between surviving and neighboring whisker projection, their death would form 'soft', in somatotopically organized inter-correlated whisker ganglia which would be tangle, inter-whisker and whisker ganglion cells should have central projection patterns that are mirror images of each other; one should resemble a 'honeycomb', the other should resemble barrels. To test this, inter-whisker surfaces were selectively denervated at birth (by electrolytically the skin between rows of whiskers) and their central projections stained by galain histochemistry (White et al., Dev. Brain Res. 72, 93) on postnatal days 3 or 6. Alternate sections were stained for cytochrome oxidase to reveal whisker patches. At both ages, galain immunoreactive terminals were sparse in brainstain whisker regions, although they occurred most often between whisker patches, sometimes to the extent that the stained fibers produced a honeycomb pattern. However, in normal adults, primary afferents with inter-whisker receptive fields had collaterals that usually ended in whisker patches; on rare occasion they terminated between whisker patches. Thus, staining patterns in the adult, showed no-natal hypothesis.

Supported by NIH DE07734, DE07662, NS17631.

694.3 ANATOMICAL AND FUNCTIONAL REORGANIZATION OF THE CUNEATE NUCLEUS AFTER NEONATAL FORELIMB REMOVAL IN THE RAT. R.D. Lang, B.L. Killackey, and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699 and Dept. of Psychology, Univ. of California, Irvine CA 92717.

Most previous anatomical studies have shown that large caliber primary afferent fibers exhibit only limited central sprouting, after peripheral nerve damage in either animal or human. Leister and Marfurt (1984) have reported that forelimb removals were carried out in newborn rats that were not to the time of adulthood, and used in terminal anatomical or physiological experiments. Sciatric nerve afferents to the brainstem were labelled with a combination of WGA-HRP and 6-8-10 rats and 8 animals that sustained neonatal forelimb removals. All of the 8 neonatal rats and 4 of 6 adult rats had dense HRP labelling that extended out of the graile nucleus into the cuneate nucleus. This implicated multi-unit recordings demonstrated the presence of hindlimb-related activity in the cuneate nuclei of all manipu lated rats. Single unit recordings demonstrated the existence of the neurons (49/ of 49 recorded that responded to hindlimb stimulation) in the cuneate that responded to hindlimb stimulation. These results are consistent with the conclusion that low-threshold, hindlimb-related primary afferents innervate the cuneate nucleus after neonatal forelimb removal and make functional contacts with neurons in this nucleus.

Supported by NS 28888 and DE 07734

694.4 EFFECTS OF FORELIMB DAMAGE AT DIFFERENT AGES ON PEPTIDE DISTRIBUTION AND LECTIN BINDING IN THE CEREBRAL DORSAL HORNS OF THE RAT. K. J. Rhoades, C. A. Bennett-Clarke, and R. W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699.

Rats that sustained forelimb removal on either enbroynic day (E-) 16 or the day of birth (P-0), or transaction of the brachial plexus as adults had sections through the cervical dorsal horn stained for galain, colliculin gene-related peptide (GCRP), or the lecin, Banderia simplicifolia-I (BS-1) 35 days after these lesions. There were age-related differences in the effects of peripheral nerve damage upon each of these markers. Damage to the brachial plexus in adulthood caused a significant increase in the density of galain immunoreactivity in the medial portion of layers I and II and an increase in the appearance of galain immunoreactivity in layers II, III and IV of the cervical dorsal horn. Such lesions resulted in significant reductions in the density of BS-1 immuno reactivity in layers I and II and decreased BS-1 binding in lamina II. Forelimb removal at birth resulted in no significant change in the density of galain immunoreactivity in layers I and II, but in the appearance of galain-immunoreactive fibers in layers III and IV. Neonatal forelimb removal resulted in no significant change as the density of GCRP immunoreactivity in layers I and II, but in a significant reduction in BS-1 binding in the medial portion of lamina II. Forelimb removal on E-16 increased galain immunoreactivity in layers III and IV, but had no effect on either GCRP or BS-1 binding in the cervical dorsal horn.

Supported by NS 28888, DE 07734, and DE 07734.

694.5 CHROMOTOPIC ORGANIZATION OF PRIMARY AFFERENTS IN THE RAT'S TRIGEMINAL SPINAL TRACT. R. S. Crissman, P. A. White, and R. W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699.

Trigeminal (V) ganglion cells with different somatotopic features of different birthdates. Larger neurons positive for neurofilament protein (NF) and which give rise to large born early in V ganglion neurogenesis with 27.3% born on E-10.5, 30.3% born on E-11.5, and 42.4% born on E-12.5. No clefts, CCRP-positive ganglion cells are born late, 51.5% on E-13.5 and 42.1% on E-14.5. The difference in birthdates for these two groups of ganglion cells is reflected by the position of their axons in the spinal tract. NF-positive axons are concentrated in the upper part of the spinal tract, but are dense in its deeper portion. In contrast, CCRP-positive axons are largely restricted to the superficial portion of the tract. Ganglion cells that bind the lectin Bandeiraea simplicifolia-I (BS-1) are also born late, 41% on E-11.5, 50% on E-12.5, 5% on E-13.5, 1% on E-14.5, and 2% on E-15.5. These are restricted to a small portion of the spinal tract. This observation is consistent with the conclusion that low-threshold, hindlimb-related primary afferents innervate the cuneate nucleus after neonatal forelimb removal and make functional contacts with neurons in this nucleus.

Supported by NS 28888 and DE 07734

694.6 IMMATURE THALAMOCORTICAL SYNAPTIC RESPONSES IN NEONATAL MOUSE BARREL CORTEX. A. A. Agmon and Diane K. O'Dowd. Deps. of Anatomy and Neurobiology and Developmental and Cell Biology, University of California, Irvine, CA 92717.

We have extended our previous study of the development of thalamocortical synaptic responses in mouse barrel cortex (J. Neurophysiol. 68, 345, 1992) to include the first 3 postnatal days (P0-P2) and the deep cortical layers. Using the thalamocortical slice preparation, whole-cell recordings were obtained in all layers in all cortical layers, and synaptic currents evoked by electrical stimulation of the thalamocortical pathway. To study the morphological basis of these responses, selected cells were stained with Lucifer Yellow, and in the same slices, thalamocortical axons were stained with DiI(5). Synaptic responses were evoked in deep (layers V/VI) neurons as early as P0, and in the incipient layer IV by P2. Responses were exclusively excitatory; they were highly labile and were often irreversibly depressed after a small number of stimulations. Current-voltage relationships indicated that the dominant component in these responses was mediated by non-NMDA glutamate receptors. In P2 animals, a single electrical stimulus to the thalamus evoked a prolonged sequence of small synaptic events lasting up to 400 ms, apparently due to temporally-dispersed transmitter release. Supported by NS03099.
694.7 PRENATAL DEVELOPMENT OF THE RAT CORTICOTRIGEMINAL TRACT. D.A. Randell* and W.E. Keenan, Division of Gastroenterology, Henry Ford Health Sciences Center, Detroit, MI 48202.

The role of the TRN as a model of somatosensory development and plasticity for over two decades. It is interesting to note, however, that most investigations have focused entirely on the ascending trigeminal pathways, with little attention paid to the maturation of the descending inputs to the primary somatosensory nuclei or the developmental interactions between the ascending and descending projections. We have addressed this issue by using the carboxycyanine dye Dil to study the prenatal development of the corticotrigeminal afferent projections to the rat. Cells of DI were placed in the region of the barreled field of trigeminal complex tissue from rats that had been sacrificed on embryonic (E) days 18 and 20 and postnatal (P) day 0 (day of birth). Numerous labeled CT axons were clearly visible in the ipsilateral somatosensory cortex at E18. At this age, CT fibers could be seen approaching the medial border of the rostral ipsilateral trigeminal brainstem complex (TBNC), but no terminals were identified within the confines of the trigeminal subnuclei. At E20, however, numerous CT axon terminals were visible in the rostral ipsilateral TBNC. A lesser number of axons were also identified in the ipsilateral TBNC. In addition, labeled axons were seen in the medial portion of the contralateral TBNC. The disposition of labeled CT axons in P0 animals was similar to that noted in adult animals, with the density of the terminals in the contralateral TBNC exceeding that seen in the ipsilateral brainstem. These results indicate that the CT pathway in the rat develops in an ipsilateral-to-central, rostral-to-caudal sequence in the late prenatal period.

Supported in part by DE07734.


In the developing somatosensory neocortex, granule cell dendrites come to be largely confined to individual barrels by selective pruning and reorientation relative to thalamocortical patch boundaries. In the developing VPM thalamus, the barrel field is occupied by DI fiber arbors (Baker et al., Soc. Neurosci. Abstr. 1994, #3). To study this issue in trigeminal nucleus principalis (PrV), the barrel link in the whisker-barrel neuronal axis are reconstituted in postnatal days 0-4. Single PrV cells were stained with Lucifer yellow in aldehyde-fixed transverse slices and the resulting labeling and whisker-patch-related autoradiograms and manually and by computer microscopy. The extent to which a single cell's dendritic tree was confined to its patch of origin was determined by computer-assisted merging of the dendrite and patch images. We find that all P0, VPM, most dendrites do not span their "home" patch. Those data suggest that PrV dendrites develop according to a different set of rules than those operating in the VPM thalamus and layer IV of barrel cortex. This may reflect the fact that primary afferent projections are quite mature in the newborn PrV when large numbers of PrV cells have yet to do so. We tested the hypothesis that PrV dendrites are a different function than VPM dendrites in adults. NIH DE07734, NS17563.

694.11 THE TIME-COURSE OF PLASTIC CHANGES IN THE BARREL CORTEX OF ADOLESCENT RATS. S. Glazewski* and K. Fox, Dept. Physiology, University of Minnesota, MN 55455.

Long-term deprivation (60 days) of all but the D1 vibrissa in adolescent rats (P28), results in expansion of the D1 representation in layers II/III of the barrel cortex. To test how rapidly this expansion occurs, we assayed plasticity in rats deprived for 5-7 days and compared the results with those obtained from rats deprived for 60 days. Two kinds of deprivation were used: one group had their vibrissae trimmed every day (VT), the other group had their vibrissae carefully pulled out every two days (VP). The receptive fields of 298 layer II/III cells located above barrels immediately surrounding the deprived barrel were measured using post-stimulus time histogram analysis. We found that functional plasticity occurred in both long- and short-term deprived animals but that the underlying processes were different. Short-term deprivation resulted in suppression of deprived vibrissa input (0.55±0.17 spikes per stimulus versus 0.45±0.15 and 0.42±0.15 in adults). These results suggest that attenuation of deprived vibrissa input might be a prerequisite for expansion of the spared vibrissa representation. Supported by NS27759.


We know that daily whisker trimming from birth alters the receptive field size and character of many trigeminal (V) barrel cells without affecting primary afferent responses. Using in vitro carbocyamine dye oxidase staining patterns, or barrel somatotopy (Jacquin et al., J. Comp. Neuroal, '94). Deprivation-induced response alterations may then reflect disrupted inhibitory mechanisms, the preservation of prenatally developed altered V barrel circuits due to altered activity-based competitive interactions, use-dependent plasticity of existing circuits, and/or an indirect effect of different whisker-related circuits that are imposed on V barrel neurons by more effective cortico-V projections. The latter is particularly compelling because the patterning and extent of the cortico-V projection is altered by V nerve injury at birth, and cortical inputs contribute to V barrel receptive fields in normal rats. To further evaluate the cortical substrate hypothesis, we performed experiments on 1-2 month old rats whose mystacial whiskers were trimmed daily from birth, WGA-HRP injections were placed in the barrel cortex labeled cortical fibers whose extent, density and pattern were normal in ipsilateral and contralateral V brainstem nuclei. However, the incidence of cells in V nucleus interpositus that were discharged by electric shocks in the contralateral internal capsule was significantly higher. This suggests that whisker deprivation impacts on "stable" aspects of cortico-V circuits. To determine whether these changes are prompted by the brainstem or cortex, slow-release polymers containing TTX are being applied to the newborn cortex. Effects on the cortico-V projection will be reported. Supported by NIH DE07734.

694.10 PLASTICITY AT THALAMOCORTICAL SYNAPSES REVEALS A SYNAPTIC BASIS FOR DEVELOPMENTAL CRITICAL PERIODS. M. C. Crair* and R. C. Malenka, Departments of Psychiatry and Physiology, University of California, San Francisco, CA 94143.

It has long been suspected that there could be a synaptic basis for the developmental critical period seen in the cortical representation of the sensory periphery. We present evidence that LTP and LTD-type phenomena might be responsible for one such developmental critical period in the somatosensory cortex of the rat. Using an in vitro thalamocortical slice preparation we tested the lability of monosynaptic response in the barrel cortex of rats to stimulus of the somatosensory thalamus and found that under a pairing protocol (depolarization to -10 ± 5 mV with 100 stimuli at 1 Hz using a blind whole-cell patch), animals beyond the critical period did not display significant plasticity (1 out of 10 cells from animals P8-P14 potentiated more than 10%), whereas thalamocortical synapses in younger animals potentiated easily (150 ± 20% in 9 out of 10 cells from animals age P3-P7). Elucidation of the mechanisms responsible for this developmental switch would aid not only our understanding of critical periods per se, but also to the growing body of evidence suggesting that synaptic plasticity commonly seen in vitro may have important in vivo ramifications as well.


We have examined the metabolic activity and size of the adult rat primary somatosensory cortex (S1) after neonatal eye removal to ask whether altered neural activity affects the growth of the neocortex. Activity in S1 was analyzed by comparing the cortical vascularization of enucleated rats with those rats that were reared with intact eyes. Cortical growth was determined by direct measurement of S1 and its component parts. The overall density of blood vessels in the enucleated animals was 14% greater than the primary somatosensory cortex, and 16% greater within the cortical barrels that represent the whisker pad. In parallel with this change, measurements of S1 and its component parts in the enucleated animals showed that those regions with increased levels of ongoing activity were larger than their counterparts in the littermate controls. Thus there was an increase in the average size of both areas S1 and of the barrels that contribute to the cortex (20%) in the experimental rats. This correlation of increased metabolic activity and cortical growth provides evidence that neural activity stimulates the elaboration of cortical circuitry during postnatal development. Such activity-modulated cortical growth during maturation may be the mechanism by which early experience encodes information in the developing central nervous system.
SOMATOSENSORY DEVELOPMENT

694.13
THE EXPRESSION OF DIFFERENT CYTOCHEMICAL MARKERS IN NORMAL AND AXOTOMIZED NUCLEUS GRACILIS PROJECTING DORSAL ROOT GANGLION CELLS IN THE ADULT RAT. J.E. Persson*, B. Lindbl, B. Råde, B. Robertson*, C. Rivera-Medina*. N.E. Jessen† and T. Skrede. Dept of Anatomy and Histology, University of Oslo, Oslo, Norway. The aim of this study was to analyze expression of various markers in normal and axotomized nucleus gracilis projecting dorsal root ganglion cells. To analyze expression of such markers we used the nuclear translocation assay. The ganglia were retrogradely labeled with Fluoro-Gold (FG) and analyzed immunohistochemically for their expression of neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), and carbonic anhydrase (CA). We found that the expression of these markers was different in normal and axotomized nucleus gracilis cells. In normal cells, NSE and GFAP were expressed at a similar level, while CA was expressed at a lower level. In axotomized cells, NSE and GFAP were expressed at a higher level, while CA was expressed at a lower level. These results suggest that the expression of these markers is regulated by axotomy.

694.14
POSTNATAL DEVELOPMENT AND PLASTICITY OF TERMINALS AND SYNAPSES IN SUPERFICIAL MEDULLARY DORSAL HORN: STEROID ANALYSIS. J.P. Golden*, J.A. DeMarco, P. Robinson and M.F. Jacobson. Anat. & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63110. Mature receptive fields in the superficial dorsal horn develop later than in other somatosensory areas. This development is associated with the presence of a critical period for deafferentation-induced plasticity. To further our understanding of the role of these observations, EM stereochemistry was used to develop connections and morphology in lamina I and II of the superficial dorsal horn in neonatal and adult rats. At birth, postnatal (P) days 1, 4, 17, and 90, we observed synapse morphology and structural changes in the superficial dorsal horn. These changes were not observed in the adult rat.

694.15
ALTERNATE PATTERNS OF EYELID MORPHOGENESIS FOLLOWING LESIONS OF THE TRIGEMINAL GANGLIA. B.L. Mungas* and I.M. Cook. Department of Anatomy, University of Tasmania, Hobart, Tasmania, 7001. In order to test the hypothesis that the alteration of the normal nervous system plays a role in the development of ocular dermal derivatives, the left trigeminal ganglion was cauterized in opossum pups at day 1 post-natal. Morphogenetic abnormalities were compared to the equivalent stages of normal development. Neurectomies were carried out using procedures described by Morohunfola et al. (1992 The Anat. Rec. 222, 599). Mother and pups were anesthetized with halothane (Keller et al. 1988 Lab. Anim., 22, 269). Pups were taken for histologic analysis 2-20 days following surgery. Pups were given intraperitoneal nebulin, and fixed in formalin, serial sectioned and stained with silver. The development of the eyelid neural non-neural constituents occurs post-natally and the lower eyelid lags behind the upper eyelid. Eyelid innervation precedes mesodermal maturation and hair follicle formation suggesting a possible neural role in cutaneous differentiation. Trigeminal gangliosic lesions only involved the sensory component of the nerve sparing the motor root. Trigeminal ligation resulted in altered spatial and temporal patterns in the eyelid that included abnormal eyelash symmetry, delayed follicle formation deletions of eyelashes and delayed dermal maturation. Sham operated pups (skin cultured but lacking a neural ligation) showed normal development in all parameters of eyelid morphogenesis. These findings support the earlier findings of Morohunfola et al. (1992 & a b), based on lesion of lombo-sacral dorsal root ganglia and spinal cord. The present study involves a purely sensory lesion and clearly implicates axons in the formation of the targets in skin, i.e. the cutaneous appendages.

694.16
ISOLATION AND CHARACTERIZATION OF THE CHICK NMDA RECEPTOR cDNA. L.K. Garner*, A. Shalash, B. Mendelev* and B. M. Davis. Dept. of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington KY 40535. A chick brain cDNA library was screened using a 3P radiolabeled oligonucleotide that corresponds to the first transmembrane region of the rat NMDAR1 subunit. A fragment (approx. 0.5 kb) was isolated and subcloned into pGEM 4Z and used for double stranded sequencing (United States Biochemical). Preliminary results show that the first 200 nucleotides of the isolated fragment are homologous (80%) to the rat and human NMDAR1 subunit at the 5' end of the open reading frame. This region corresponds to the amino terminal end of the NMDA receptor and codes for the extracellular domain that precedes the first transmembrane region. Comparison of the chick cDNA to other NMDA subunits shows no homology. This cDNA fragment has been used to make cRNA probes to determine the normal ontogeny of the NMDA receptor mRNA in the chick spinal cord. Preliminary in situ hybridization results show high levels of the NMDA receptor mRNA located primarily in the dorsal horn of the spinal cord (stage 39, E13). We are currently determining the distribution of NMDA receptor mRNA in the spinal cord following administration of kainate and various NMDA receptor antagonists.

694.17
TEMPERATURE-DEPENDENT BEHAVIORAL CHANGES AND CEPHALIC WOUNDS IN CAPSACIN NEONATALLY TREATED RATS. P. Carrillo*, M. Canacho, M. Salas and P. Pacheco, NFE, Unv. Veracruzana; Centro de Neurobiologia e Inst. de Invest. Biomédicas, UNAM, México, D.F., México. Neonatal administration of Capsaicin (CAP) in the rat interferes with eye-opening, scratching frequency and is also associated with skin ulcers emergence. Here we investigate, a) if the wounds emergence is a trophic effect or is produced by the increased cephalic scratching and, b) how the behavior of CAP rats was modified by the warm environmental exposure. We used control and neonatally (day 1 p) treated (60 mg/kg) and adult male rats. In experiment a) rats were using (n=20) or not (n=20) a plastic collar fitted over the nose to prevent head scratching. The control group (n=10) were not disturbed. In experiment b) the behavioral patterns of control or experimental rats were visually recorded at 24 °C or 35 °C of temperature (120 measurements/30 min). Both CAP groups the head wounds appeared around the 23 days of age and the progression in the number and extension of the lesions was also similar. Data indicate that wounds emergence is not provoked by the increased cephalic scratching. Moreover, a significant reduction in self grooming frequency (p<0.05) and relaxed extended posture (p<0.01) in CAP animals was observed. Data showed that hyperactivity related with trophic skin lesions and has also a long term interference with the behavioral regulatory mechanisms to heat exposure. SUPPORTED BY CONACyT (PGR)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
SYNAPTIC VESICLE PROTEIN 2 (SV2) LOCALIZATION IN THE DEVELOPING HAMSTER PRIMARY VISUAL PATHWAY.

Aiming to better understand the role of NMDA receptors in synaptic plasticity, researchers used the SV2 monoclonal antibody, 10H1, to examine 2-D Western blots containing metabolically labeled rapidly transported proteins of the hamster retinal ganglion cell axons. They observed changes in SV2 expression during development, with SV2 levels increasing in the superficial retina (SC) and decreasing in the deep retina (DC) of the newborn hamster. This study highlights the dynamic regulation of synaptic proteins during the early stages of neuronal development.

FUNDATIONAL FUNCTIONALITY OF OPTIC AXONS REGIMENTING INTO THE PRIMARY OPLAJECTORY CENTER OF THE PRIMATE. P. Scalisi, R. Hoffer, A. Lostoc, and V. Lammert, Dept. of Anatomy, SUNY, Health Science Center at Brooklyn, Brooklyn, New York 11203 and Dept. of Anatomy, College of Optometry, The City University of New York, 11203.

This study investigates the role of optic axons in guiding the development of retinal ganglion cells. The researchers found that optic axons play a crucial role in regenerating fibers and forming a coherent terminal field in the primate olfactory cortex. This work contributes to our understanding of the complex interactions between axonal growth and target identification in the developing nervous system.

NEONATAL ADMINISTRATION 5,7-DIHYDROXYTRYPTAMINE RESULTS IN SYNTACTIC REORGANIZATION IN THE SUPERFICIAL GRAY LAYER OF THE HAMSTER PRIMARY COLLICULUS. D. A. M. Mooney and R. W. Rhoades, Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43614.

Neuronal administration of 5,7-dihydroxytryptamine (5,7-DHT) in neonatal hamsters results in a selective lesion of serotonin (5-HT) in the superficial gray layer of the hamster's superior colliculus. This study demonstrates the potential for synaptic reorganization following chemical lesions and suggests a role for 5-HT in shaping neural connectivity in the developing brain.

EFFECTS OF NEONATAL 5,7-DIHYDROXYTRYPTAMINE TREATMENT ON RETINAL GANGLION CELL AND OPTIC NERVE FIBER NUMBER IN THE ADULT HAMSTER. J. C. Creighton, W. M. H. Van der Hoeven, R. D. G. S. Van der Hoeven, and R. W. Rhoades, and D. A. M. Mooney, Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43614.

This study investigates the long-term effects of 5,7-DHT treatment during neontal development on retinal ganglion cell and optic nerve fiber number in adult hamsters. It demonstrates the lasting impact of early lesioning on the number of neurons and axonal growth, providing insights into the critical period for synaptic development.

NMDA RECEPTOR BINDING IN TECTUM OF DEVELOPING XENOPUS. S. Uhid* & L. G. Murakami, Dept. of Physiology, SUNY, Buffalo, NY 14214.

This study explores the development of NMDA receptor binding in the tectum of developing Xenopus laevis. The researchers found that NMDA receptor number changes during postnatal development, which may correlate with changes in synaptic plasticity and axonal growth. This work contributes to our understanding of neuronal development in an amphibian model system.

THE ROLE OF RETINOCOLICULAR AXONS IN THE DEVELOPMENT OF THE CORTICOTECTAL PROJECTION OF MICE. M. Khachab and I. L. Bruce, Dept. of Biomedical Sciences, Creighton University, Omaha, NE 68178.

This study investigates the role of retinocollicular axons in the development of the corticotectal projection in mice. It demonstrates that the development of this projection is closely linked to the growth and refinement of retinal ganglion cells, highlighting the importance of axonal guidance cues in the developing visual system.

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695.7


NMDA receptor activity is essential in the plasticity of the developing frog retinotectal projection. Ca2+ entering through the NMDA receptor may act through a Ca2+-CaMKII-CaMKII (CAMK) pathway. We are starting to explore this possibility by combining recombinant viral technology with in vivo imaging techniques. Vaccinia virus containing the DNA sequences encoding truncated, constitutively active CAMKII and D-galactosidase (βGal) was injected into the retina of stage 46-47 tadpoles. Intense infection throughout the CNS, as indicated by the expression of βGal, occurred by day 3. To examine RGC axon morphology, a small amount of smooth green fluorescent protein (GFP) was injected into the retina of stage 46-47 tadpoles. Animals were screened the next day to select those with a single brightly labeled RGC axon. Three days later, when tadpoles were stage 48, this newly emerged axon was observed in vivo by using a confocal microscope. The extent of viral infection was verified by checking βGal expression. Single axons were reconstructed. In the animals infected with βGal and constitutively active CAMKII was expressed, the average total length of 13 RGC axons was 373 ± 49 µm (±SE) and the average number of axon branches was 13 ± 2. These values are smaller than those in the control animals with no virus injected (n=15) and those infected with βGal Virus (n=12), where the average total axon length was 523 ± 67 and 616 ± 116 µm, the average number of axon branches was 27 ± 3 and 24 ± 2, respectively. Thus constitutively active CAMKII expressed in the tectal cells reduced the RGC axon total length and the branch number in 3 days. These results indicate that CAMKII activity in the postsynaptic tectal neurons is likely to be involved in the control of the growth of presynaptic RGC axons. We are taking time-lapse images of growing axons to study the mechanism by which their morphology is modified.

695.9

EFFECTS OF SEROTONIN ON PATCH-CLANPED NEURONS IN THE OPTIC TECTUM OF THE FROG. Malavey A.A. and E.A. Debski. Sch. of Bio, Sciences, University of Kentucky, Lexington, KY 40506.

Recent immunocytochemical studies have shown that serotoninergic tectal ganglion cells project to the optic tectum of frogs (Liu & Debski, J. Comp. Neurol. 338:361-404, 1993; Liu & Debski, Neurosci. Abstr. 19:452, 1993). The role of serotonin in the functioning and/or development of the tectum is unclear. There are few external studies of the effects of serotonin on tectal cells in tadpoles and juvenile bullfrogs by applying whole-cell patch-clamping techniques to brain slices. Perfusion of the slices with 100 µM serotonin induced a sustained outward current of 3.0±2.0 PA/PF (n=9) in 27% of the tadpole cells voltage-clamped at -60 mV. Under these conditions 50% of the cells recorded in juvenile frogs to date have shown this serotonin-induced current (n=10, 1<57±1:85 PA/PF). Blocking the membrane potential to between -50 and 0 mV from a holding potential of -70 mV revealed serotonin-induced outward current in 50% of the tadpole cells and in 75% of the juvenile frog cells. Preliminary experiments conducted to address the possibility that exposure to the cells serotonin resulted in a cell hyperpolarization of approximately 10 mV. In addition to an evoked outward current, in some cells serotonin also increased the frequency of synaptic inputs. Cells that responded to serotonin also responded to NMDA. External application of 33 µM NMDA in the presence of TTX resulted in the activation of an inward current at a holding potential of -40 mV. Our data suggest that serotonin may modulate NMDA receptor activity in the optic tectum by changing the potential of the membrane upon which these NMDA receptors sit. Supported by NSF grant IBN-9209651.

695.10

THE DEVELOPMENT OF NORMAL AND ABNORMAL GENICULOCORTICAL TOPOGRAPHIES IN THE SYRIAN HAMSTER. A.L. Smith, K.Koga and J.D. Thompson (SPON: Brain Research Association). Laboratory of Physiology, Parks Road, Oxford, OX1 3PT, UK.

The orderly mapping of the lateral geniculate nucleus (LGN) onto area 17 in the adult hamster is disrupted by unilateral eye removal on the day of birth. Ipsilateral to the remaining eye, single injections of tracer into an area of cortex which receives input from one eye can produce dual focal of retrogradely labelled geniculate neurones (Trewavas & Thompson, 1992, Eur. J. Neurosci. 4:1104). We have studied the development of both the normal and the aberrant geniculocortical projections. Separate injections of red and green fluorescent latex microspheres, spaced mediolaterally, were made in visual cortex of neonatal hamsters that had been monocularly enucleated on the day of birth (P0). No topographic order was discernable in animals injected on P2 when a single cortical injection labelled neurones throughout the LGN. With two injections, the populations of red and green labelled cells were spread completely. At P6, cortical injections antilateral to the remaining eye produced an essentially adult pattern; most of the LGN was unlabelled and there were distinct roid of red and green labelled cells. The pattern in the ipsilateral geniculate was different with more overlap between the populations of red and green labelled cells; however, the projection was much more restricted than that seen at P2. The characteristic double focus seen in adult differentiated LGNs appeared later.

The emergence of geniculocortical to topography between P2 and P6 in the hamster coincides with the invasion of cortical pial by the majority of geniculate axons, as revealed by transneuronal tracer. Preliminary experiments placing MK801-containing Elavax on visual cortex failed to disrupt this early phase of topographic refinement.

This work was supported by the Wellcome Trust, UK.

695.11

THE DEVELOPMENT OF THE RETINO-COLLICULAR MAP IN THE SYRIAN HAMSTER. L.D. Thompson and P.M. Cordery. (SPON: Brain Research Association). Laboratory of Physiology, Parks Road, Oxford, OX1 3PT, UK.

Previous studies applying DII to the retina of fetal rats revealed considerable topographic disorder in the retinal projection to superior colliculus (SC; Simon O'Leary, 1992, J. Neurosci. 12:1212). We have used two retrograde tracers, red and green fluorescent latex microspheres, to examine the development of topography in the retino-collicular projection of the hamster. Small (25-50 nL) injections of tracers were made in all four quadrants of the SC in the neonatal rats. Both the injection and the orientation (rostral/medial) of the paired red-green injection sites was varied. Single injections of tracer on P2 produced labelling over approximately one third of the retina, even though the injection occupied a very much smaller area of SC. When injections were separated by 400 µm or less and irrespective of their orientation, there was considerable overlap in the populations of red and green labelled cells. Larger separations resulted in very little overlap between the red and green labelled populations of ganglion cells whereas similarly large rostrodorsal separations produced much less overlap. Similar numbers of ganglion cells were labelled following injections into caudal or into rostral SC. By P6, single injections labelled ganglion cells in much more restricted regions of retina and pairs of injections separated by 500-1000 µm produced little or no overlap. Older animals displayed increasing precision in the retino-collicular projection such that, at P21, injections separated by less than 200 µm could produce two distinct layers of labelled ganglion cells.

These results reveal that a crude retinal topography exists for the mediolateral axis of the SC in neonatal hamsters and that the overall topography refines during the period of ganglion cell death.

695.12


We have found that there are four major events in the functional development of the neonatal rat superior colliculus. (1) Spontaneous discharges are the earliest recordable activity, becoming manifest on postnatal day 5 (P5). (2) The first evoked response to electrical stimulation of the optic nerve is detectable on P10, and (3) the first flash evoked responses in the superior colliculus occur on P12/13. (4) On P14/15, eye opening occurs. Since spontaneous activity occurs first and may influence the other developments, we sought to determine the extent to which each of these events may influence the others. We measured the frequency of spontaneous spikes at different postnatal age; interval histograms were constructed between P5-P15. Preliminary data, based on 28 animals, suggest an interesting trend. On P6, P8, P10, and P13, there are infrequent distributions of intervals, indicating clustering of shorter intervals and a relatively high frequency of spontaneous discharges during these ages. These trends may be physiologically important, as the flash and the flash evoked responses in the superior colliculus are 24-36 hours. Our data suggests that there is a temporal increase in spontaneous activity coinciding with the development of new functional capability. To further characterize the source of the spontaneous activity, we injected 2% xylocaine into the optic nerve head, silencing optic nerve activity at P6-P11. These results indicate that spontaneous discharges in the superior colliculus are independent of the retina.

Supported by grants from NATO, NSF and USARC.
695.14 GLYCOEN IN THE VISUAL SYSTEM OF YOUNG AND ADULT FERRETS. Kathryn Hermann*. Lab. Neurophysiology, NIMH, Poolesville, MD 20837, USA.

It is well established that glycine is an inhibitory neurotransmitter in the brainstem and spinal cord. Glycine, however, is also present in retinal ganglion cells both in adult and developing ferrets, and glycine receptors are abundant in the early postnatal mammalian neocortex. In the present study the lateral geniculate nucleus (LGN) and the visual cortex were examined in adult and developing ferrets for the presence of glycine employing immunohistochemical procedures using a rabbit anti-glycine antibody. In adults, glycine positive cells were seen in the lateral geniculate and visual cortex. In adults, immunopositive cells in the LGN were mainly large or medium sized neurons, and glycine positive cortical cells were found in all layers, but occurred in greater frequency in the intragranular layers. By birth, glycine-positive cells were already plentiful in the LGN. In the developing cortex, however, glycine-positive neurons were sparse in the cortical plate, although abundant in the subplate (SP) and marginal zone. In addition, the SP/intermediate zone contained a dense plexus of glycine-positive fibers, perhaps representing thalamocorticical axons. To determine whether the glycine is seen in relay neurons of the LGN, I injected HRP and/or fluorescent latex beads into visual cortex of adult (~P65) ferrets. Most if not all retrogradely labeled LGN cells were also glycine positive. Since the relay neurons of the LGN are likely to use an excitatory neurotransmitter, the presence of glycine in LGN projection neurons strongly suggests that glycine within thalamocorticical axons acts on the glycine site of the NMDA receptor, mediating the appropriate excitatory thalamocortical response. The presence of glycine in these axons might indicate an additional role for glycine perhaps as a neurotrophic factor.

695.15 SIGNAL TRANSMISSION IN THE LATERNAL GENICULATE NUCLEUS OF MONKEYS WITH SURGICALLY INDUCED ESOTROPIA. E.L. Smith III*, Y.M. Chino*, H. Cheng*, Y. Sasaki*, M.J.C. Crawford†, R.S. Hanworth† and G.K. von Noorden‡. College of Optometry, University of Houston.§ University of Texas at Houston†. Department of Ophthalmology, Baylor College of Medicine, Houston, TX.

Early strabismus, a form of abnormal visual experience which often leads to permanent vision anomalies, produces dramatic alterations in the response properties of neurons in the monkey's visual cortex. In the present study, the morphology and laminar distribution of LGN neurons are clearly altered by strabismus, but it is not known whether the functional status of LGN neurons is also affected. We investigated the effects of early strabismus on the transmission properties of LGN neurons surgically induced esotropia by using microelectrode recording techniques to compare the responses of individual LGN neurons with those of their retinal inputs (G-potentials) and LGN action potential pairs (n=65). We measured orientation, spatial frequency, temporal frequency and contrast response functions using drifting sine-wave gratings. Contrary to our previous findings in strabismic cats, there were no obvious differences between the transfer characteristics of control- and treated-eye neurons. The results suggest that, in comparison to the cat, the physiological status of the primate LGN is less susceptible to environmental influences.

695.16 THE EFFECT OF NEONATAL MONOCULAR APHAKIA OR OCCLUSION ON THE EXPRESSION OF CAT-301 ANTIGEN, PARVALBUMIN AND CALBINDIN IN THE LATERAL GENICULATE NUCLEUS OF MACAQUE MONKEYS. N. Jain*, J. McKee†, V. Rema†, and H. Kogo*. Dept. of Neurology and Inst. for Developmental Neurosciences, Vanderbilt University, Nashville, TN, and Yerkes Research Center, Emory Univ., Atlanta, GA.

Macaque monkeys (Macaca mulatta) were reared from birth to about one year of age either with surgically induced monocular aphakia, resulting in a blurred retinal image, or with a black occluder on one eye, which prevents visual experience. Levels of expression of the Cat-301 antigen, parvalbumin (PV) and calbindin (CB) were examined with immunohistochemical techniques in the lateral geniculate nucleus (LGN). Both types of deprivation resulted in a marked reduction of Cat-301 staining in both parvocellular and magnocellular LGN layers connected to the deprived eye, with deprived magnocellular layers retaining a relatively higher level of staining than the deprived parvocellular layers. PV and CB immunoreactivity, however, was reduced only moderately in the deprived layers, with a more pronounced reduction ipsilateral to the deprived eye. These results show that changes in the expression of the Cat-301 antigen, and PV and CB are similar, whether produced by aphakia or occlusion. Remarkably, alterations can be more pronounced in the layer receiving inputs from ipsilateral than the contralateral deprived eye. (Supported by NIH EY02668, EY09573 and RR-016E).

TRANSLATION VI


We examined motor control in normal and shiverer (shv) mutant mice using the rotarod assay, a forced motor activity which tests for balance and coordination. Shiverer mice carry a deletion in the myelin basic protein (MBP) gene, resulting in CNS demyelination and characteristic motor dysfunction. Shiverer mice were evaluated at 6-7 weeks of age in order to avoid the toxic micronucleation in older shv mice, and all animals were acclimated to the test apparatus prior to testing at 3 pm and 6 pm each for 1 min. Homozygous mutant mice had a significant increase in mean fall time (0.05±0.6, n=4) relative to wild-type and heterozygous (shv+) mice (n=110.11, n=4). Comparable results were obtained at speeds of 12 and 18 rpm, 1 min each. Non-acclimated animals showed learning curves with progressive improvement in performance when tested daily for four days, although the shv/shv animals consistently had more falls than normal control animals.

We also examined the motor assay for the retinal output of morphology in the performance of shv/shv animals in a paradigm of challenging tasks. Rotarod day 2 animals received thamic transections of either of reticuloticollidofondosecre-type 2neaectomy (5-2-2A) neurons, or of the o-2a (luteal cell) glialis (G-2-4) cells. Shv/shv transplanted recipients showed a significant improvement in rotarod performance (172±2.5 falls, n=18) although they continued to demonstrate a gross phenotype, indicating that the recovery measured in the retarded represents a multiplicity of functional recovery. Immunohistochemical analysis of tissue harvested after rotarod analysis revealed the expression of MBP in the CNS of transplanted shv/shv animals. We included control animals in the rotarod performance relative to non-transplanted shv/shv mice (9.8±1.8 falls, n=8). RNA analysis of two of these animals showed no evidence of MBP transcripts, consistent with the interpretation that the thamic transplantation failed. Our results demonstrate that the rotarod is a sensitive measure of motor function in demyelinated mice, and suggest that transplantation can lead to an improvement in motor function in these mice. The cell and vascular basis for the motor recovery in shv/shv recipient transplants remains to be elucidated.

696.2 KINEMATIC AND EOG ANALYSES OF CONTROL AND SPINAL CORD TRANSECTED RATS DURING TREADMILL LOCOMOTION. J.W. Boston*, X.M. Xu, M.B. Bunge, and B. Callanas. The Miami Project to Cure Paralysis and the Department of Neurological Surgery, University of Miami, Miami, FL.

We previously demonstrated that mild-thoracic spinal cord transections bridged by Schwann cell-filled guidance channels open at both ends (OPEN CHANNELS) promoted a greater number of axons across the transected site than open cord transections closed at the distal end. The following studies were undertaken to determine if behavioral or electrophysiological correlates of this increased axial growth exist.

Unoperated control rats were studied to determine the normal walking pattern in a spacious environment. These data were then compared to spinal cord transected, closed channel and OPEN CHANNEL groups. Rats were studied 1-4 weeks post-surgery. For the kinematic analysis, animals were taken at 30 frames/sec during 7.5-30s of regular forward movement while rats were suspended in a sling over a treadmill which was moving at 0.05-0.15m/s. In conjunction with the kinematic study, EMG experiments were done, in which periods of activity ranging from 7.5-120s were collected from forelimb and hindlimb muscles, digitized at 200Hz, rectified, and analyzed. OPEN CHANNEL rats displayed significantly more hindlimb EMG than did rats with closed channels. Further, alternating hindlimb movements were observed in rats with OPEN CHANNELS at some treadmill speeds, but which deteriorated at lower or higher speeds. To determine if this movement was under some voluntary control, we also calculated the cross-covariance functions of forelimb vs. hindlimb movements (kinematic analysis) and muscle activations (rectified EMG analysis).

Because involuntary rhythmic movements can confound the validity of overly sensitive behavioral measures of motor function, this analysis may prove useful in the assessment of the consequences of spinal cord transection. In the assessment of motor function, movement after complete or incomplete spinal cord injury.
606.3 EFFECTS OF FETAL SPINAL CORD (FSC) IMPLANTS AND EXERCISE ON MUSCLE ATROPHY IN CHRONIC SPINAL RATS. N.B. Repper*, J.D. Houli, C.A. Peterson, C.M. Guifery, C.L. Perry, F.D. Sweatman and E. Garcia-Rill, Dept. of Anatomy and Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205

The potential for surgical and pharmacological procedures to influence the characteristic atrophy of hindlimb skeletal muscle following spinal cord injury was examined. Adult Sprague-Dawley rats sustained a complete spinal cord transection (T4) by aspiration of intrathecal clamps. Four groups of animals were prepared: T4 rats exercised on a treadmill, T4 rats exercised following FSC injection; T4 rats transplanted with FSC tissue, T4 rats transplanted with FSC tissue and exercise. FSC tissue was transplanted into the anterior (T) muscles were prepared for measurement of cross-sectional area of individual myofibers or for immunocytochemical analysis of myonuclei恕fibers or for immunocytochemical analysis of myonuclei by immunocytochemistry. Results: T4 rats lost 76% of their motor nerve axons, while type Ila (fat, fast) fibers were reduced and type Ila (fast, fatigue resistant) fibers were more prominent. These results suggest that the shift in expression towards type IIb fibers caused by spinal cord Tx is reduced by exercise by increasing the proportion fast IIb fibers. Supported by NSF Grant RII 8922103 and NIH Grant NS 29328.


Traumatic conduction injury to the human spinal cord most frequently occurs at the cervical level and produces varying degrees of chronic tetraplegia. The deficits in the hands and arms persist in part from cervical nerve degeneration. Using our adult rat model of incomplete cervical SCI and forelimb behavior tests, the present study begins investigations of motor neuron replacement after cervical SCI as a means of ameliorating forelimb dysfunction. Cervical SCI was produced by dropping a 10 gm weight 2 cm onto the C7 spinal cord segment. Between 1-4 weeks post-SCI, SCI rats (n=11) had significant forelimb deficits compared to sham-injured rats (n=4) as shown by 1) decreased grades for proximal (elbow) and distal (wrist and digits) extension and flexion (grasp) with a modified Tarlov scale and during Forelimb Placing Tests, 2) reduced forelimb grip strengths in the Grip Strength Test, and 3) reduced pellet retrievals and well clearance scores in the food retrieval Staircase Test. At 5-6 weeks, CNTF (Regeneron Pharmaceuticals, Inc., 5 μg/1) were administered at the C7 segment of SCI rats through an intrathecal cannula. At 5 weeks, some SCI rats received transplants at C7 of dexamitino-yellow labeled, E14-15 rotoral spinal cord cell suspensions (1.05 X 10⁷ viable cells) incubated for 60-90 min in 10.5 μg CNTF (n=3) or saline (n=2). At 10 weeks, forelimb function of all SCI rats had not improved. Nissl-stained cells with motor neuron morphology and ChAT-immunoreactive cells were found in small similar numbers in all transplants. The results show that grafted transplanted motor neurons needs to be further examined. Supported by The Miami Project to Cure Paralysis.

606.5 EFFORTS TO REINNerve ADULT RAT GASTROCNEMIUS MUSCLE BY EMBRYONIC SPINAL MOTOR NEURONS TRANSPLANTED INTO PREDEGENERATED TIBIAL NERVE. D.E. Eric, L. Lippincott and R.P. Bunce. The Miami Project to Cure Paralysis, U.A. of Miami School of Medicine, Miami, FL 33136

Motor innervation is required for effective contraction and maintenance of skeletal muscle. If motor innervation is lost the muscle becomes denervated and undergoes progressive atrophy which ultimately leads to degeneration. Efforts to illicit muscle contraction or significantly retard muscle atrophy by direct electrical stimulation of the muscle are relatively ineffective without a nerve supply. Previously, we have reinervated denervated adult rat gastrocnemius muscle by transplanting a heterologous nerve graft. A population of dissociated adult rat spinal cord, into the adult rat tibial nerve, which was then transplanted proximal to the transplant site (Exp. Neurol. 193:124:372-376). Currently, we seek reinervation by transplanting into neonatal (P1) and 3 weeks after atomy. Within six weeks posttransplantation large multipolar cells, resembling alpha motor neurons, were observed within the transplant site. Axons originating from the transplanted cells were identified within the nerve stump and within the previously denervated gastrocnemius muscle. Transplanted motor neurons survived up to 4 weeks (the longest survival) and could be retrogradely labeled with the tracer fast blue. This study demonstrates that embryonic spinal motor neurons, transplanted into the predegenerated adult peripheral nerve, are able to survive and extend axons into the denervated target muscle. This approach may provide the innervation necessary to artificially control muscle contraction by functional electrical stimulation, thus making previously unavailable therapies available to the muscle. (The Helfield Foundation, The Miami Project to Cure Paralysis, NRS 59144-01).

606.6 TRANSPLANT MEDIATED BEHAVIORAL RECOVERY OF WEIGHT SUPPORT & INTERLIMB COORDINATION IN NORMAL, SPINAL & SPINAL-TRANSPLANT RATS. D.Y. Myria*, B.Clarke, A.Tessler and M.Murray. Dept. of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129

Transplanted fetal spinal cord tissue into verified lesion sites can support locomotion in newborn spinal rats (Howland et al.,1993). Surgical procedures and a battery of quantitative behavioral tests were developed to extend these results to a neonatal rat model. Blocks of E14 cervical spinal cord tissue were placed into the newly transected rat thoracic spinal cord on the day of birth. Reflex and locomotor behavior was studied in normal rats, spinal rats and spinal rats from E14 ventral spinal cord, 4 weeks of age. Tests examined posture, weight support and interlimb coordination and included reflex bipedal and quadrupedal treadmill locomotion, locomotion over a variety of terrains and stair climbing. Transplanted animals demonstrate coordination and weight supported steps on all types of locomotion but also exhibit marked hypermetria in the hindlimbs (HLS). EMG recordings taken from each limb during locomotion also demonstrate brainstem mediated spinal cord graft in transplanted, but not spinal rats. Spinal rats demonstrate coordinated HLS stopping only during tests of supported, reflex bipedal locomotion. Stair climbing, a task requiring complex postural adjustments and adaptive changes in weight support and interlimb coordination, was completed differently by each of the 3 groups of trained rats. Supported by NIH NS 24707 and VA Research Service.

606.7 PHARMACOLOGICAL MODIFICATION OF TRANSPLANT MEDIATED BEHAVIORAL RECOVERY IN NORMAL, SPINAL AND SPINAL-TRANSPLANT RATS. M.Murray*, A. Tessler, K. Simanys and D.Y. Myria. Dept.of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129

Fetal tissue transplanted into spinal cord will promote development of locomotor function in cats (Howland et al., 1993) and in rats transected as neonates (Myia et al., this volume). Specifically, interlimb coordination across the gait phases occurring in transplanted but not untreated, rats, shown by both behavioral and physiological methods. Transplants may improve interlimb coordination by promoting connectivity across the lesion, by increasing weight support or spinal mechanisms operating at the regional level or by a combination of both mechanisms. Because regenerating 5-HT axons have been shown to cross grafts in neonates, descending serotoninergic systems might contribute to behavioral recovery. Trained normal, spinalized and transplanted rats were studied for the effects of serotonergic agonists. Quadrupedal treadmill locomotion was videotaped both before and after drug administration and analyzed. The non-specific 5-HT receptor agonist quipazine, produced a marked increase in the step cycle duration and augmented the amplitude and duration of hindlimb extension, flexors and axial muscles in transplanted animals. Quipazine did not affect either normal or transected rats at the doses used indicating that the descending 5-HT system may be involved in transplant enhanced locomotion. Immunochemical and fluorescent results also indicate the possible contribution of both the descending 5-HT and NE systems in the improved function. Supported by NIH NS24707 and VA Research Service.


Our previous work has indicated that pain sensitivity can be reduced by transplanting adrenal medulla into the rat spinal subarachnoidal space. To further elucidate the mechanisms of this phenomenon, we examined several characteristics of tyrosine hydroxylase (TH) mRNA and preproepinephrine (PPEnK) mRNA were investigated using in situ hybridization in rat adrenal medullary explants in culture. A large number of TH mRNA-expressing precursors were identified in all cultures. 32P-labeled oligonucleotide probes and anti-TH monoclonal antibody applied to 15-day cultured sections. The relative mRNA levels and intraganglionic mechanisms were quantified by image analysis. Both TH mRNA and PPEnK mRNA levels in explants started to increase after 4 days in culture, and peaked a week at 7 days. At 14 and 28 days in culture, levels of TH and PPEnK mRNA decreased, but were still significantly higher than that of intact normal adrenal medulla and then at 1 and 4 days in culture. The TH mRNA significantly increased by 1 day, and continued to increase up to 4 days in culture. Thereafter, it remained at a level similar to adult adrenal medullary explants, adrenal medullary transplants contained numerous TH mRNA labeled cells at 1, 2 and 12 weeks after transplantation. However, only a few cells expressed PPEnK mRNA by 2 weeks post-transplantation. These results indicate that, while surviving chromaffin cells can still synthesize catecholamines when transplanted to the CNS, the synthesis of opiod peptides may remain low. In addition, these findings suggest that the regulatory mechanisms of TH mRNA and PPEnK mRNA expression in rat adrenal medullary cells differs under the environmental influence of culture and the CNS. Supported by NIH grant NS25064.
XENOTRANSPLANTATION OF ENCAPSULATED BOVINE CHROMAFFIN CELLS FOR THE TREATMENT OF INTRACTABLE PAIN: A PHASE I CLINICAL STUDY

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Chromaffin cells are known to release a cocktail of alkaline substances such as catecholamines and opioids. The response of these cells to the administration of allogeneic and xenogeneic chromaffin cells has been shown to alleviate pain in rodent models. To circumvent the problem related to the shortage of human cadaver donors, we are investigating the possibility of transplanting encapsulated xenogeneic chromaffin cells in humans suffering from intractable pain. Chromaffin cells are isolated surgically from the adrenal gland and encapsulated in a tubular (5cm long) 950μm OD semipermeable membrane which allows diffusion of nutrients and neurotransmitters but prevents rejection of the graft by the host immune system. Bacteriologic analysis and capsule catecholamine release studies were performed before implantation.

Ten patients suffering from chronic pain related to advanced cancer or deafferentation were transplanted with cell loaded capsules in the lumbar subarachnoid space (n=8) or lateral ventricle (n=2) using minimally invasive techniques. No evidence of serious complications or significant host tissue reaction related to the capsules was observed. Viable cells positive for tyrosine hydroxylase and melanin-enchephalin immunostaining were observed in all but one of the nine explanted capsules. Seven of the ten patients showed improvement in their chronic pain on the basis of decreased narcotic intake (4/9 opioid responders) or improvement in pain scores (3/9 non-responders to narcotics).

POLYMER-ENCAPSULATED CELLS GENETICALLY MODIFIED TO SECRETE HUMAN NGF IMPROVE COGNITIVE FUNCTION FOLLOWING INTRAVENTRICULAR IMPLANTATION

Neurosurgery Laboratory, University of California, San Francisco, CA, Neuro2A culture, Transgenic mice, Gene Therapy.

Exogenous NGF has been reported to improve cognitive function in rats with age-related cognitive deficits. However, the long-term effectiveness of exogenous NGF to treat human cognitive disorders have been expressed about the risks involved with supplying NGF to the CNS. Recent evidence in our laboratory suggest that encapsulated, genetically modified NGF-secreting cells may be delivered into the mouse brain, thereby preventing the deleterious effects of the surgical procedure and the side effects of autologous NGF in the recipient's CNS. As a proof of concept, we have tested the potential of exogenous NGF-secreting cells to ameliorate some age-related cognitive deficits in a transgenic mouse model of mental impairment. Exogenous NGF-secreting cells were encapsulated in semipermeable membranes, and implanted stereotactically. NGF production was 10% of that previously reported. The results showed that these encapsulated cells transplanted into the hippocampus of aged mice significantly improve the long-term memory and learning ability of the transgenic mice compared to the control group. This result suggests that NGF-secreting capsules can be used to treat age-related cognitive impairments and that the NGF-secreting cells are able to migrate to the site of the implantation and release NGF to the brain tissue. The results are promising and suggest that encapsulated NGF-secreting cells may be a potential therapy for age-related cognitive impairments.
696.15 Host Brain Modulation of Intra-Retrosplenial Transplants of CholinergicSeptal Neurons. Yong J. Lue, and Walter C. Loev, Dept. of Neurosurgery and Physiology, and Program in Neuroscience, University of Minnesota Medical School, Minneapolis, MN, U.S.A.

Previous studies from our laboratory have demonstrated that cholinergic-rich grafts derived from fetal septal nucleus can re-establish the cholinergic innervation in the retrosplenial cortex (RSC) of rats with lesions in both forebrain and cingulate pathways. Concurrently, a significant amelioration of spatial memory deficits in these animals was observed. In the present study we postulated that the grafts can become functional interconnected with the neural circuits of the host brain, and can thus be regulated by activity from the host brain. To test this hypothesis we evaluated the effect of the animals' performance in a 6-ram radial maze task on high affinity cholinoxygen (HACU). Three groups of rats were used: 1) animals with control RC (NC), 2) rats with lesions of the fornix and cingulate pathways, and 3) lesioned rats with fetal septal grafts in the RSC (RSCsep-TPL). Animals in the NC group were further divided into two subgroups: 1) rats that performed the maze before the determination of HACU levels (BEH), and 2) rats that were kept in their home cage (NON-BEH) and paired with a BEH mate for HACU assessment. In NON-BEH animals, the HACU values (pg/mg/min/mg protein) were 21.2 ± 0.9 (mean ± SEM) for the NC, 9.9 ± 1.0 for the FX, and 17.7 ± 0.7 for the RSCsep-TPL animals. The BEH subgroups had HACU values of 29.8 ± 1.8 for the NC, 19.1 ± 1.0 for the FX, and 22.9 ± 0.9 for the RSCsep-TPL. In comparing HACU levels with their NON-BEH counterparts, t-test indicated significant increases in the NC (p < 0.001) and RSCsep-TPL (p < 0.005) groups, but not in the FX animals (p = 0.565). These results suggest that fetal septal graft in the RSC can become functionally interconnected with the host neural circuitry, and that the activity of the implanted cholinergic neurons can be modulated by the host brain. (Supported by NIH grant RO1-NS-24464).


Excitative neurotoxin ibotenic acid (IB) or kainic acid (KA) injection in the brain in one way causes death of extantic neurons, but also induce a neurotrophic environment for neuronal growth. The lesion effects when confined in a tract, provides a trophic throughway for the axonal outgrowth of transplanted neurons and reinervation of 5-7 mm distal target in 3 weeks. Spague-Dawley rats with fetal nigral dopamine neurons (6-OHDA, 12 ± 5ug/ml) in medial forebrain bundle. 1-2 weeks later, KA (1ug/ml) or IB (10x20ug/ml), or phosphate buffer (PB) were microinjected through a glass pipette to a 5 ± 7mm long track between nigra and striatum, followed by graft of E14 embryonic cells into nigra. Animals were either perfused and stained with tyrosine hydroxylase immunocytochemically (TH-im) 3-4 weeks later, or injected with HRP in the striatum and their brains were stained 24 hours later in the graft regions. The 6-OHDA degenerated DA neurons in nigra and completely denervated the striatum. (n=13). Unilateral turning was evident after amphetamine challenge. Transplanted cells of nigral grafts grew distinctly in a bundle along the entire length of KA/IP tract (n=5), but not into the PB tract (n=5). The TH-im fibers did not leave the KA tract when coursing through the globus pallidus, but quickly spread out when reaching the striatum. Reinnervating TH-im fibers were distributed in patches in the striatum near pallidum but non-discerningly in rostral striatum. HRP tracing reveals labeled neurons in the graft (n=3). Behavioral studies show that amphetamine challenged unilateral turning was reduced by about 40-60% after the bridgeing method of transplant.

696.18 PROTEOLYTIC ENZYMES INVOLVED IN GLIAL CELL MIGRATION FOLLOWING TRANSPLANTATION OF FETAL RABBIT BRAIN TISSUE INTO MOUSE BRAIN. M.K. Del Bino*, F.L. Tchclingerian, C.M. Jacob, INSERM U134, Hospital de la Salud, Paris 75651 France.

Following grafted of fetal (E25) rat brain fragments into the striatum of shiverer mouse (a mutant lacking MBP) brain. Glial cells migrate predominantly along white matter tracts. Differentiated astrocytes can be identified by the anibody ThyGAP1 which recognizes rabbit but not mouse GFAP. Transplanted oligodendrocytes produce proteolytic enzymes which contain MBP. Immunoelectron microscopy shows that transplanted astrocytes are well integrated into host brain in both perivascular and perineuronal positions. To study the role of proteolytic enzymes in the migration of transplanted glial cells 3 days to 4 weeks after transplantation we used immunohistochemistry and non-radioactive ISH. Transplanted astroglial cells are often immunoreactive for tissue plasminogen activator (tPA). Some immature transplanted cells at the graft nidus show urokinase PA-like immunoreactivity. In shiverer mouse, reactive astroglia exhibit iPA and inflammatory cells show UPA. Immature transplanted cells express the mRNA for matrix metalloproteinasises (MMP) type 1 and type 3 for up to 4 weeks. Transient expression of MMP3 is occasionally observed at a distance from the graft sites associated with cell migration. We conclude that some proteolytic enzymes may be associated with the migration of transplanted glial cells. However, they are not unambigious identifiers of migrating glial cells.

AGING PROCESS

697.1 CHRONIC ADMINISTRATION OF GM1 GANGLIOSIDE ENHANCES CHOLINERGIC PARAMETERS IN THE AGED BRAIN. M. Hadjiconstantinou*, T.G. Fong and N.H. Neff. Deps. of Psychiatry, Pharmacology and The Neuroscience Program. The Ohio State University College of Medicine. Columbus, Ohio 43210

Although the mechanism(s) of action of GM1 ganglioside is unresolved, it has been postulated that it might enhance the action of endogenous trophic factors by augmenting their synthesis and/or release, and by altering trophic factor receptor characteristics. We now provide evidence that GM1 has a synergistic action with NFG in the brain of old animals. Chronic intracerebroventricular administration of GM1 increases choline (Ch) uptake and choline acetyltransferase (ChAT) activity in the striatum and hippocampus of old rats. When a low ineffective dose of GM1 (0.5 mg/kg for 4 wks) is co-administered with various doses of NFG (0.2, 0.5 and 1 pg/day for 4 wks) we observe an enhancement of the GM1 effect on Ch uptake and ChAT activity. Our results are consistent with apparent potentiation by GM1 of the effect of NFG on cholinergic parameters in aged animals.
697.4 METAL-CATALYZED OXIDATION OF BOVINE NEUROFILAMENTS (NF) IN VITRO. JG Trapp*, JH Kim*, AC Costello*, and GWW Johnson. Departments of Pathology and Neurology, Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD; *University of Alabama, Birmingham, AL.

NF are important determinants of the shape and size of nerve cells. The oxidation of NF, potentially relevant to aging, neurodegenerative disorders, and Wallerian degeneration, has not been studied. In this investigation, we combined biochemical and ultrastructural methods to study the metal-catalyzed oxidation (MCO) of bovine NF using an associatedFe(III)-O2• system. The oxidation parameters are being determined by significant increases in carbonyl content, which were time and concentration dependent. Polyacrylamide gel electrophoresis and immunoblot analyses revealed fragmentation of oxidized NF proteins, predominantly NF-H and NF-M. Electron microscopy showed that oxidized NF formed dense aggregates and bundles of laterally aggregated filaments. Finally, we also demonstrated that oxidized NF proteins were more susceptible to catalytic proteolysis. In view of the growing evidence to support increased oxidative damage as a factor in aging, the mechanism by which metal-catalyzed oxidation of NF causes aggregation or fragmentation remains to be elucidated.

697.5 CYTOSTATIC-INDUCED ASTROCYTE HYPOXIA: ROLE OF PEROXISOME PROLIFERATOR-ACCTIVATING RECEPTOR-RELATED DEPENDENT-RELATED DEPENDENT MEDIATORS. E.A. Bloemendaal, J.E. De Vrije, J.A. van den Bosch, J.M. Mork, and E.M. Schipper, Dept. of Neurology and Neurosurgery, Maastricht Univ., Maastricht, The Netherlands, and Aging, Jewish General Hospital, Montreal, QC, Canada, H3T 1E2.

Background: Compound, cytostatically (CSH), induces gliosis in situ and the appearance of peroxidase-positive (dys)regulation in the cultured astroglia. Gliosis identical to gial inclusions which progressively accumulate and are evident in the cells. In addition, CSH-treatment protects cultured astroglial from subsequent H2O2 and mechanism-stress activation. Objective: To determine whether changes in antioxidant defense system are present in CSH-treated astrocytes. Methods: and Results: CSH treatment (880 μM) induced a 5-fold increase in glutathione (GSH) concentrations and a persistent augmentation of manganese superoxide dismutase (MnSOD) activity in cultured astrocytes. In contrast, astroglial catalase and increase activities redox state in oxidative stress. Lipid peroxidation was suppressed by CSH treatment while copper-zinc superoxide dismutase activity remained unchanged. Glutathione peroxidase activity was increased after CSH-treatment and declined thereafter to below control levels. Conclusions: Elevations in intracellular GSH concentrations and sustained augmentation of MnSOD activity may be responsible for astrococyte hypoxia. CSH exposure to CSH-treated astrocytes may serve as a useful model for stress-related (Idiopathic) and other oxidative stress in aged. In the latter, astrocyte hypoxia may facilitate the establishment of reactive gliosis in the face of concomitant neuronal depletion.

The aim of this study was to explore if paranodal axon Schwann cell networks (ASCNs), which are entities assumed to take part in removal and degradation of worn-out organelles in large myelinated PNS axons, can serve as deposits for indigestible materials with aging. We addressed this issue by examining the occurrence of autofluorescent lipopigments in large myelinated motor and sensory axons of rats aged 2-25 months. After fixation with 4% paraformaldehyde, 10 µm thick longitudinal sections were cut from the lumbar ventral (VR) and dorsal (DR) roots and examined with a Zeiss fluorescence microscope equipped with phase-contrast optics and a FITC filter set. From each root, 200 randomly selected selected paranode regions in nerve fibres 8-12 µm in diameter were searched for autofluorescent granules. Orange-yellow granules situated in close apposition to the paranodes in small paranodes was observed in both VR and DR axons from 3-4 months of age. Both the percentage of paranodal regions of this type and the number of granules increased with age. Up to 1 year of age, VR axons showed the highest granular contents. From this age, >90% of the paranodes in both the VR and DR showed autofluorescent granules. Some paranodes in the oldest animals contained >15 granules, which generally appeared in clumps and often were of larger sizes in VR than DR axons. In both axon types, the vast majority of the granules were situated distal to the nodal mid-level. EM analysis showed that most granules were lipofuscin bodies associated with ASCNs. The progressive age-related accumulation of indigestible materials within ASCNs may serve a function to shield the aging perikarya mainly of motor neurons from being crammed with retrogradely transported worn-out organelles from the axon.

697.10 REGRESSIVE CHANGES IN STEROID ACCUMULATION IN AGING ANDROGEN-SENSITIVE RAT SPINAL NUCLEI. M.R. Wildes, A.P. Fine and D.P. Sengupta*. Program in Neural Science, Indiana University, Bloomington, IN 47405.

Neurons in the rat spinal nucleus of the bulbocavernosus (SNB) are sensitive to androgens throughout life. Androgen titers decline with normal aging in most mammals and are coincident with concomitant regressive changes in SNB dendritic length, soma size, and target muscle weight. Using steroid autoradiography, we determined whether regressive changes in the ability of these neurons to accumulate steroids might also occur in aging.

Male rats (12, 19 and 25 months old; n=4 per age) were castrated 48 hours prior to injection with total testosterone (1.5 g/kg body weight) and killed 1.5 hours later. Accumulation was assessed in the steroid sensitive, sexually dimorphic SNB and dorsolateral nucleus (DLN), as well as in the non-sexually dimorphic retrotrapezoid nucleus (RTN) from each rat (at least 50 cells per nucleus). The number of SNB and DLN motoneurons reaching criteria (Poisson) was reduced by as much as 21% at 25 months, while no changes were observed in RTN. The density of labeling also declined with age in all motor nuclei and was particularly pronounced in the SNB. At 12 months of age, 55% of SNB motoneurons were labeled at more than 8 times background levels, but less than 30% were labeled at this density by 25 months. Thus, declines in androgen accumulation by steroid-sensitive motoneurons reflect age-related changes in circulating androgen titers. Regressive changes in motoneuron morphology could result from a reduced ability of androgen to act directly on these motoneurons. (Supported by NIH AG02909)}
698.1

TWO LOCi ENCODE SNAP-25 IN ZEBRAFISH AND GOLDFISH.
C. Riesinger, E. Salamek, and D. Larhammar. Dept of Medical Genetics, Box 365, Uppsala University, S-751 23 Uppsala, Sweden.

SNAP-25 (synaptosome-associated protein of 25 KDa) is a presynaptic protein involved in vesicle docking and release (Sümer et al., Nature 365, 1993), as well as neurite extension (Oan-Sand et al., Nature 364, 1993). We have previously shown that SNAP-25 has retained highly conserved from mammals to Drosophila (Riesinger et al., JBC 268, 1993). We initially isolated cDNA clones for SNAP-25 from goldfish because we intended to study the roles of this protein model systems in the regulation of nervous system of this species. Although SNAP-25 is evolutionarily conserved we discovered sequence variability within the goldfish. This was unexpected, but similar to the situation in Drosophila: a gene duplication early in fish evolution, leading to SNAPA and SNAPB, followed by the tetraploidization 15-20 My ago (Riesinger and Larhammar, PNAS 90, 1993). However, we found no homologues of SNAPA and SNAPB, which is less appropriate for molecular genetic studies. Instead, the closely related diploid species provides an excellent system for an comprehensive study of the functions of SNAPA and SNAPB. The predicted protein sequences are highly similar between zebrafish, goldfish, and rainbowfish. Similarly, between zebrafish and mammals. Interestingly, the zebrafish clones reveal two alternative splicing variants of exon 5. The existence of these two variants have previously been shown for chicken (Bark, JMB 233, 1993) and humans (Bark and Wilson, Gene 139, 1994). The expression of SNAPA and SNAPB and the alternatively spliced exons will be studied in zebrafish.

698.2

THE MAMMALIAN SEC1P-HOMOLOGUE IS A CHROMAFFIN GRANULE-BINDING PROTEIN. T. Schaefer, A. Hodel, C. Haus, A. Matus and M. B. Burren. Friedrich Miescher-Institute, PO Box 2543, CH-4002 Basel, Switzerland.

Membrane proteins of the synaptic vesicle and the presynaptic plasma membrane together with soluble proteins form a secretory fusion complex conserved from yeast to neurones (Niswander et al., Cell 92, 318-324). We have localized three of these membrane proteins in chromaffin cells, which secrete catecholamines stored in granules. Synaptins (1A and 1B) and SNAP-25 are found in a plasma-membrane-containing fraction, whereas VAMP/synaptobrevin is concentrated on the granules. Recombinant synaptin 1A has been used in an affinity chromatography assay to isolate synaptotagmin, a receptor protein of the type 2 aminophospholipid translocase. Solubilized granule membranes contained a single protein with high affinity for synaptin 1A, the mammalian homologue of Sec1p, mSec1 (Hodel et al., J Biol Chem. 269, 8623-8635). In yeast, this hydrophilic protein acts late in the secretory process. Genetic suppressor analyses predicted the interaction of Sec1p with Sec21, a yeast homologue of synaptin 1A, and with Sec9, a homologue of rab3A (Aalto et al. (1993) EMBO J. 12, 4095-4104). Although rab3A is present on chromaffin granules, we did not detect it bound to synaptin 1A together with mSec1. Western blot analysis of subcellular fractions revealed three pools of mSec1 in chromaffin cells: on plasma membrane vesicles, in the cytosolic protein fraction, and on the chromaffin granule membranes. mSec1 can be detached from this latter by high salt treatment. It binds back to its receptor upon addition to granules at physiological salt concentrations. The characterization of both the attachment of mSec1 to the granule and its binding to synaptin 1A should be interesting for the interpretation of neurotransmitter vesicles with the plasma membrane in docking and fusion.

698.3

UNIFORM QUANTAL RELEASE MAY BE REGULATED BY THE SYNAPSE-SPECIFIC CLATHRIN ASSEMBLY PROTEIN F1-20/AP-3.
W. Ye and E.M. Leter.* Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, and Institute of Biotechnology, University of Tennessee Space Center, Huntsville, TN, USA.

We reported the characterization and cloning of the synapse-specific phosphoprotein F1-20. We overexpressed F1-20 and, reported that F1-20 is the clathrin assembly protein. At presynaptic terminal, clathrin mediated endocytosis is involved in the biogenesis and recycling of synaptic vesicles. Here we characterized the ability of bacterially expressed F1-20/AP-3 to bind to and assemble clathrin coated vesicles. We find that bacterially expressed F1-20/AP-3 can bind and assemble clathrin as efficiently as preparations from the bovine brain. This establishes that the clathrin assembly activity found in F1-20/AP-3 preparations from brain extracts is indeed encoded by the cloned gene for F1-20. We also demonstrate that post-translational modification is not required for activation of the clathrin binding or assembly function of F1-20/AP-3. Ultrastructural analyses of the clathrin cages assembled by bacterially expressed F1-20/AP-3 reveals a strikingly narrow size distribution. Because mature synaptic vesicles are derived from precursors in coated vesicles, we hypothesize that an important function of the synapse-specific clathrin assembly protein F1-20/AP-3 is to limit the range of vesicle sizes, and therefore increase the uniformity of quanta release. We also expressed the 33 KD NHE-terminus of F1-20/AP-3 in E. coli, and measured its ability to bind and assemble clathrin. We find that the 33 KD NHE-terminus of F1-20/AP-3 constitutes a clathrin binding domain. We found that while the bacterially expressed 33 KD NHE-terminus of F1-20/AP-3 binds to clathrin triskelia, it fails to bind to preassembled clathrin cages and is not sufficient for clathrin assembly.

698.4

CELLULAR AND SUBCELLULAR LOCALIZATION OF SYNTAXIN IMMUNOREACTIVITY IN THE RAT CORTICAL AND STRIATUM.
S.R. Sesack* and C. L. Snyder. Departments of Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.

Syntaxin is a membrane protein that concentrates in axon terminals and may participate in synaptic vesicle docking prior to Ca2+-mediated fusion events. We sought to characterize the cellular and subcellular distribution of immunoreactivity for syntaxin in the rat forebrain using both an antibody directed against a bacterially expressed syntaxin protein (Bennett et al., Science, 257:255) and a monoclonal antibody against the identical membrane-derived protein, HPC-1 (Barnstable et al., Dev. Brain Res., 20:286). Whether labeled by immunoprecipitation or from brain ultrathin sections, HPC-1 proteins were localized exclusively to axons and terminal varicosities in the rat dorsolateral striatum and frontal cortex. Immunoeactive terminals made exclusively asymmetric synapses, primarily on dendritic spines, while unlabeled terminals in the adjacent neuropil made either symmetric or asymmetric synapses on somadendritic targets. Immunogold labeling for syntaxin or HPC-1 was localized primarily to non-synaptic regions of the plasma membrane. These results suggest that the syntaxin immunoreactive synapse is an integral membrane component of a transmitter vesicle protein localized to a subpopulation of forebrain terminals that use excitatory amino acids as neurotransmitters. The apparent exclusion of syntaxin immunorelabeling from the presynaptic active zone must be considered in light of potential limitations in antibody penetration or epitope recognition at junctional specializations. Conversely, the non-synaptic localization of syntaxin implies that its functional role may not be limited to synaptic vesicle docking. This work was supported by USPHS grant MH50314.

Synaptic vesicle (SV) docking is thought to be mediated by the binding of two SV proteins, VAMP and synaptotagmin, to two plasma membrane proteins, syntaxin and SNAP-25. We have identified n-sect, 1, a soluble neural-specific 68 kDa protein, and demonstrated its high-affinity binding to syntaxin (Kc17, 7 mM). This recombinant n-sect binds syntaxin in a complex distinct from the docked SV complex, based upon immunoprecipitation and glycerol gradient centrifugation studies. N-sect binds VAMP and SNAP-25, so leading to syntaxin immobilized on agarose beads. In the absence of n-sect, VAMP binds the syntaxin 1 and 4 (but not 2 and 3) isoforms. This VAMP binding to syntaxin 1 (but not syntaxin 4) is potentiated ten-fold in the presence of SNAP-25, suggesting a mechanism for achieving both specificity and high affinity binding of VAMP-containing SVs to the appropriate syntaxin target membrane. The proposed pathway of SV docking and fusion includes the displacement of synaptotagmin by the soluble protein tSNAP, and subsequent binding of NSF to syntaxin, SNAP-25, and VAMP. We have exploited this pathway with recombinant proteins binding to immobilized syntaxin.

DIFFERENTIAL RESPONSES OF PROTEIN KINASE C SUBSTRATES (MARKS, NEUROMODULIN, AND NEUROCRANIN) PHOSPHORYLATION TO CALMODULIN AND S100: E.-S. Shen, F.L. Huang and K.-P. Huang, NIH, Bethesda, MD 20892.

Phosphorylation of three physiological substrates of protein kinase C (PKC), MARKs, neuregulomin (Nm), and neuregulin (Ng), present in a mixture were analyzed to determine their relative efficiencies as substrates of PKC α, β, γ and δ and relative sensitivities to inhibition by calmodulin (CaM) and S100. The rationale for these experiments was to mimic the in vivo condition where these substrates and inhibitor proteins co-exist. In addition, since PKC and these substrates are all phospholipid-binding proteins, the presence of all these components and phospholipids in the same reaction mixture may create an environment to allow selectivity for each PKC isotype. Based on the kinetically sensitive Be-1,2 and Xα, the phosphorylation of each individual substrate, we estimated the order of efficacy as PKC substrate was MARKS< Nm< Ng. The rates of PKC α-catalyzed phosphorylation of Nm and Ng in a mixture containing MARKS was significantly reduced as compared to that phosphorylated individually by this isotype, compared at a higher level of MARKS. Although phosphorylation of MARKS, Nm, and Ng individually by PKC is known to be inhibited by CaM, when present in a mixture, both CaM and S100 preferentially inhibited MARKS phosphorylation over those of Nm and Ng. Protein-activated catastrophic fragment of PKC (PKM) was used to determine the effect of CaM on the CaM- and S100-modulated inhibition of PKC substrate phosphorylation. CaM and S100 inhibited the PKM-catalyzed phosphorylation of MARKS only in the presence of CaM while phosphorylation of Nm and Ng by PKM were inhibited more prominently by CaM in the absence than in the presence of CaM, StO10 was relatively ineffective in inhibiting the phosphorylation of these two substrates even in the presence of CaM. The results presented here demonstrate that MARKS is likely the most preferred substrate of PKC in the brain and its phosphorylation by PKC is most sensitive to inhibition by CaM and S100.


Purified small synaptic vesicles possess a form of Ca<sup>2+</sup>-calmodulin-dependent protein kinase II (CaMKII) which activates, as does the cytosolic form of CaMKII, most of the Ca<sup>2+</sup> sensitive activities. The interaction between synaptic-associated CaMKII and the synaptic vesicle membrane was studied using specific CaMKII affinity labeling. Purified synaptic vesicles were labeled with 3-(trifluoromethyl)-3-(methylypropyl)imidazoline ([<sup>3</sup>H]-TIM). Upon photolysis, synapsize-associated CaMKII was labeled, suggesting that part of the CaMKII is embedded in the lipid bilayer. The results of this work evidenced the presence of the CaMKII active site in the interaction. We performed liposome binding studies to determine if purified soluble CaMKII II could bind to liposomes, using a sedimentation assay. We found that soluble CaMKII II bound to sucrose-loaded liposomes and that the binding was dependent upon the presence in the liposome membrane of anionic lipids, such as phosphatidylserines. Binding of CaMKII II to liposomes was also dependent on salt concentration. We examined that CaMKII II contributes to the interaction. Soluble CaMKII II bound to liposomes was also labeled using [<sup>3</sup>H]-TIM, indicating that some portion of the protein may be embedded in the membrane. This finding provides new insight to the participation of CaMKII in nerve cell signalling.
699.1

A ROLE OF NITRIC OXIDE IN GANGLIONIC TRANSMISSION OF RAT SUPERIOR CERVICAL GANGLIA. H. Tang and N. J. Dun. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43614

Immunoreactivity to nitric oxide synthase (NOS-I) was localized to nerve fibers opposing the majority of rat superior cervical ganglion (SCG) neurons. NOS-I was either too low to be detected or absent in the postganglionic neurons. The hypothesis that nitric oxide (NO) may modulate synaptic transmission in SCG neurons was examined by evaluating the effects of agents that elevate tissue NO levels on synaptic responses and on depolarizations induced by pressure application of acetylcholine (ACh). Intracellular recordings were made from neurons in isolated rat SCG. Superfusion of L-arginine (10-300 μM) dose-dependently increased the amplitude of excitatory postsynaptic potentials (EPSPs) evoked by stimulation of cervical sympathetic trunk in approximately 50% of ganglion cells studied. The membrane depolarization induced by pressure application of ACh was also increased in some but not in all ganglion cells. D-arginine in comparable conc. caused no significant change of the EPSPs. Sodium nitroprusside (10-300 μM) reversibly increased the EPSPs in a concentration dependent manner. The present study shows that NO-immunoreactivity is present in nerve fibers presynaptic to postganglionic neurons and in SIF-like cells and that NO when released from these neural elements may potentiate synaptic transmission. (Supported by NS18710 & NS24226)

699.3


Previous results suggest that CGMP is involved in long-term potentiation (LTP) in the CA1 region of hippocampal slices, perhaps as the presynaptic modulator of a retrograde messenger (Zhou et al., 1994). Consistent with this idea, the membrane-permeable analog 8-BrcGMP also produces activity-dependent long-lasting potentiation of evoked postsynaptic currents (EPSCs) in dissociated cultures of hippocampal neurons (Arancio et al., 1993). This potentiation is paralleled by a decrease in the coefficient of variation and an increase in the frequency but not the amplitude of spontaneous miniature EPSCs, suggesting that CGMP acts presynaptically. However, a postsynaptic increase in LTP might indirectly produce presynaptic effects. To attempt to distinguish between these possibilities, we injected substances directly into the pre- or postsynaptic neuron. Injection of CGMP into the presynaptic cell paired with weak presynaptic activity produced a long-lasting increase in the amplitude of the EPSC. CGMP alone or vehicle either did not affect LTP or paired with activity produced no potentiation. Injection of CGMP into the postsynaptic cell paired with presynaptic activity also did not produce potentiation. Similarly, post-synaptic CGMP did not cause any increase in the amplitude or the frequency of spontaneous miniature EPSCs. In preliminary experiments, injection of the membrane-permeable guanyl cyclic nucleus inhibitor LY83583 into either cell block the induction of long-term potentiation by repeated tetanic stimulation. These results demonstrate that a presynaptic increase in cGMP produces activity-dependent long-lasting potentiation that may contribute to LTP.

699.2

SPERMINE AFFECTS NEUROTTRANSMISSION IN SLICES OF STRIATUM AND HIPPOCAMPUS: EFFECT OF CALCIUM. P.A. Ferchmin; D'Souza, P.G.; E.M. Rivera; Vesna A. Eterovic and T.J. Teyle. Center for Molecular and Behavioral Neuroscience, Department of Biochemistry, Univ. C. del Caribe, Bayamon, PR 00960 and Department of Neurobiology, College of Medicine, Northeastern Ohio Universities, Rootstown, OH 44272

Spermine inhibited neurotransmission in slices from rat striatum and hippocampus. In the latter, spermine increased paired-pulse facilitation of stratum pyramidal neurons to a lesser extent in a. radiation of the CA1 area. A similar pattern was observed in the presence of ω-conotoxin (ω-CTX), a blocker of voltage-sensitive Ca++ channels (VSCC). In addition, a decreased Ca++ concentration in the perfusing solution also increased paired-pulse facilitation. Spermine effect was larger at 1 mM Ca++ than at 2 mM and was almost nil at 3 mM Ca++. Addition of APV, an NMDA antagonist, did not interfere with spermine effect on paired-pulse facilitation. These results support the hypothesis that spermine is an endogenous neuromodulator that decreases the release of neurotransmitters by blocking presynaptic VSCC and perhaps also by blocking VSCC on dendrites and soma of pyramidal cells. (Supported by NSF-EPSCoR and NIH-R01 RR03053)

699.4


Many, but not all, glutamate terminals express a high affinity, Na+-dependent transporter for L-proline. Proline may play a role in synaptic transmission at these sites. This idea was tested by recording Schaffer collateral-commissural synaptic potentials extracellularly in area CA1 during superfusion of hippocampal slices with proline. Two concentrations of proline were used: a concentration typical of normal CSF (3 μM) and a concentration present in CSF of persons with hyperprolinemia type II (30 μM). Neither proline concentration altered synaptic transmission when testing was performed 15 min after its addition to the superfusion medium. However, both concentrations enhanced synaptic transmission when they were continuously present in the medium from the time of slice preparation, as indicated by a statistically significant upward shift in the slope of field EPSP slope against fiber volley amplitude. In another series of experiments, proline was added to control medium and the field EPSP was recorded for the next 4 h. In the presence of proline, EPSP slope began to increase in about 40 min and reached a plateau in 80-90 min. The plateau values of EPSP slope were 24 ± 9% and 35 ± 10% greater than baseline for 3 and 30 μM proline, respectively. In contrast, EPSP slope in control slices declined by 8 ± 10%.

These results suggest that concentrations of proline normally present in CSF enhance transmission at the Schaffer collateral-commisssural synapse. The presynaptic proline transporter may serve to regulate this process. Finally, the childhood seizures associated with hyperprolinemia type II may result from excessive facilitation of glutamate transmission.
PRESYNAPTIC INHIBITION OF INHIBITORY SYNAPTIC TRANSMISSION MEDIATED BY METABOTROPIC GLUTAMATE RECEPTORS IN MONOSYNAPTICALLY ISOLATED PAIRS OF CULTURED HIPPOCAMPAL NEURONS. R. Makl* and M.A. Dichter.1,2 David Mahoney Institute of Neurological Sciences, 1Dept. of Neurology and Pharmacology, University of Pennsylvania School of Medicine and Graduate Hospital, Philadelphia, PA 19104.

Whole-cell patch clamp recordings were performed to elucidate the effects of the metabotropic glutamate receptor (mGluR) agonist ACPD and the recently described mGluR antagonist 3-phenyl-4(carboxamido)cyclohexylglycine (MCPC) on inhibitory synaptic transmission in low density cultures of hippocampal neurons. Application of ACPD (100 µM) resulted in a reversible decrease in the amplitude of IPSCs (33.6±4.2% of control, n=200 ± 50 mM) completely reversed the effects of ACPD (n=7), MCPC alone had no effect on IPSC amplitude. Examination of miniature IPSCs (mIPSCs) indicated that changes in 0-mM pipette RMP decreased the mGluR agonist sensitivity did not account for the decrease in evoked IPSC amplitudes; therefore, inhibition of the IPSCs was due to a presynaptic mechanism. Application of D-APV (50-250 µM) led to a decrease in 8 out of 13 pairs tested; these 8 inhibitory pairs also exhibited an increase in baseline NMDA-receptor mediated noise, indicating an increase in the ambient concentration of AMPA receptor receptors. The partial agonist of L-glutamate, AP4 (Makl, Robinson, Dichter, J Neurosci, in press) we concluded that 1) all inhibitory neurons in our very low density cultures express functional mGluRs responsive to ACPD, and 2) inhibition of transmission is increased in ambient Glutamate concentration, and that this occurs only in the cases where there was a source for Glutamate. Modulation of the activation of presynaptic autoreceptors by increased ambient Glutamate concentration. The present study indicates that there is an enhancement of inhibitory synaptic transmission in the CNS. Supported by GM-34781 (MAJ) and AG-12305-01 (RM).
699.13

We previously reported that there is a large population of inhibitory receptors present on cultured rat embryonic neurons, that activated only by glycine or GABA or by either one (Neurosci. 52:83-96, 1993). Also, whole-cell current responses to bath-applied agonist have both desensitizing and nondesensitizing components, with the former being more sensitive to block by antagonists.

To determine which of these postsynaptic receptors are involved in inhibitory synaptic transmission, cultures of EPSPs (EPSPs) and MPSCs (MPSCs) were digitized at 20 Hz and analyzed using a peak detection program (Aoki, N., et al. Neurosci. Meth., in press), which calculates the peak amplitude and an exponential decay time constant (τ) for each event. Amplitude and τ were skewed, and grouped data showed no discernible peaks. The effects of different antagonists on the amplitude distributions were complex and did not provide insights concerning the involved inhibitory receptors. On the other hand, the decay distributions were consistently best fit by the sum of four Gaussian distributions (i.e. τ = 8.31 ± 0.96 s, τ = 16.5 ± 2.8 s, τ = 25.9 ± 2.3 s (n = 22) and τ = 49.3 ± 6.8 s (n = 25) ms). These 4 classes of decay times have different pharmacological sensitivities to antagonists. The results of experiments using different concentrations of strychnine and bicuculline lead to the following conclusions: τ1 is mediated by the nondesensitizing GABA-A, τ2 by the desensitizing GlyR, γ1 and γ2 by the desensitizing Gly/GABAR. However, since all four decay components are seen in cell-attached patches that exhibit MPSCs, it is possible the responses are generated by one receptor with complex binding affinities and sensitivities to block by antagonists.

699.14

Chick VIIIth nerve fibers form somatic synapses in the nucleus magnocellularis and generate large EPSCs. A previous study of brain slices showed that the neurotransmitter, presumably glutamate, activates and then desensitizes AMPA receptors (Trussell et al., Neurosc. 10:1185). The kinetics of glutamate and kainate responses in these cells are voltage dependent (see abstract by Otis, Raman, & Trussell, this meeting). We examined changes in the time course of the EPSC with postsynaptic potential to see whether voltage-sensitivity might reveal additional information about the transmitter’s lifetime and receptor desensitization. The decay of the AMPA receptor EPSC could be described by two exponentials, of 0.7-1 ms and 6-14 ms. With depolarization from -65 to +125 mV, both components slightly lengthened, but the relative magnitude of the slow component increased 5-fold, resulting a marked overall slowing of the EPSC. Such a large slow component could not be explained by a long channel burst duration or desensitization time course. The increase with depolarization in the magnitude of the slow phase is consistent with an increased receptor affinity. We propose that there is a prolonged phase of transmitter removal from the synaptic cleft. Synaptic depression, monitored with EPSC pairs, was reduced at positive holding potentials. Since receptor desensitization is reduced by depolarization, depression may in part result from desensitization, a consequence of the slow clearance of transmitter.

699.16

Basal forebrain neurons, which form the main cholinergic projection to cortical and subcortical regions of mammalian brain, receive afferents from both the sympathetic and parasympathetic nervous systems. In the present study, a thin-slice preparation was used to study the postsynaptic currents of basal forebrain neurons. Cortical sections (200 μm) were prepared from 10-14 day-old rat pups, incorporated into horizontal slices of the diagonal band of Broca and substantia innominata. Whole-cell recordings were made from visually identified magnocellular neurons, using a potassium acetate-based internal solution. Neurons with diameter > 20 μm were selected, as they stained positive with acetylcholinesterase histochemistry, and thus were considered to be cholinergic. In most cells, spontaneous synaptic responses could be recorded, displaying sensitivity to either bicuculline (8 μM) or 2-cyano-γ-4-nitrophenyl 2,3-dione (CNQX). These events were not readily blocked by tetrodotoxin (TTX). Following bilateral stimulation within a 50-100 μm radius of the recorded cell synaptic responses, both CNQX- and bicuculline-sensitive evoked responses could be recorded. The mean time constant for decay (r) for CNQX-sensitive spikes was approximately 3.03 ± 0.31 ms and was 27.5 ± 2.4 ms for bicuculline spikes (as measured at 20-23°C). Carbachol (CC): 0.3-1 μM reduced r in an amplitude dependent manner. In addition to reducing the amplitude of these postsynaptic events, inhibition by CCh was frequently observed as failures of synaptic events. In summary, spontaneous synaptic events have been preserved in thin-slice preparations and that excitatory and inhibitory afferents to the magnocellular cholinergic basal forebrain express presynaptic muscarinic receptors. [Supported by Wellcome Travel Award (W.H.G.) and M.R.C. (A.R.B.]}
699.17
SYNAPTIC CONNECTIONS BETWEEN CELL PAIRS IN RAT HIPPOCAMAL SLICE CULTURES.
D. DEBANNE*, N.C. QUEIRENO, B.H. GAHWILER and S.M. THOMPSON
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Pairs of individual neurons were recorded in rat hippocampal slice cultures with
sharp electrodes or in whole-cell configuration. Unitary synaptic
connections were studied between pre- and postsynaptic cells in CA3
and CA1 hippocampal fields by eliciting single action potentials in the
presynaptic cell. Monosynaptic excitatory connections were highly probable
between CA3 and CA1 pyramidal cells (p=0.96, n=125) with almost no feed-
forward inhibition (p=0.024). By contrast, disynaptic IPSPs, blocked by
CNQX or bicuculline, were observed with a very high probability (p=0.43)
between CA3 pyramidal cells. Monosynaptic excitation was found in 56% of
cases between CA3 and CA1 cells (n=91) but only between 26% of CA1 pyra-
midal cell pairs (n=25). AMPA- and NMDA-components of EPSPS could be
identified pharmacologically at both CA3/CA1 and CA1/CA1 cell synapses,
with kinetics similar to those seen with extracellular stimulation. Single
action potentials evoked in interneurons resulted in monosynaptic, bicuculline
sensitive IPSPS only in pyramidal cells within the same region. Paired-pulse
facilitation of unitary EPSPS and paired-pulse depression of unitary IPSPS,
were observed. Probability of transmitter release, studied in recordings
where failures could be unambiguously distinguished from spontaneous or small
PSWs, was found to be close to 100% at both excitatory and inhibitory
synapses. Bath application of the inhibitor of glutamate release, alenosine,
reduced the amplitude of unitary EPSPS in a graded manner, indicating
that the release at excitatory synapses is multiquantal.

699.19
CHARACTERIZATION OF INTRACELLULAR CALCIUM OSCILLATION IN RAT HIPPOCAMAL NEURONS IN THE PRESENCE OF MAGNESIUM IONS. T. TANAKA*, H. SATO, and
N. MATSUKI. Dept. of Chem. Pharmacol., Fac. of Pharmac. Sci., Univ. of
Tokyo, Tokyo 113, Tokyo
It is widely known that the embryonic neurons can develop
morphologically and functionally in culture and form the synaptic
network. In Mg²⁺-depleted condition the cultured neurons show
spontaneous and periodical oscillations of intracellular Ca²⁺
concentration ([Ca²⁺]), which are synchronized among the cells. In the present study the spontaneous oscillatory changes of [Ca²⁺], were observed even in the
presence of physiological concentration of magnesium and characterized pharmacologically. The hippocampal cells from embryonic day 18 rats
were cultured for 10-15 days and changes in [Ca²⁺] of single cells were
measured by a microfluometric technique with fura-2. The spontaneous
oscillation was blocked completely by application of 1 μM tetrodotoxin.
CNQX (10 μM) and nicardpine (10 μM) blocked the oscillation completely while APV showed only a partial depression of the increase in [Ca²⁺]. This contracts with the results in Mg²⁺-free condition, in which either APV or CNQX alone inhibits the oscillation completely. The inhibitory neurotransmitter GABA (10 μM) suppressed the spontaneous oscillation, whereas bicuculline (3 μ M), a GABAₐ antagonist, slightly
enhanced the amplitude. Basic fibroblast growth factor (bFGF) also had
depressant effect on the oscillation, and this effect was encountered by
suramin. These results suggest that excitatory and inhibitory transmission
is involved in the formation and maintenance of the intracellular Ca²⁺
oscillation.

700.1
GABA-MEDIATED OUTWARD CURRENTS IN HERMISIDENM PHOTOCEPTEOR'S. E. YUZENGA* and A. KUZING. Marine Biological Laboratory,
Woods Hole, MA 02543.
GABA is the only inhibitory neurotransmitter at the mechanonervous-hair cell-photoreceptor
synapse in Hermisenda. In addition, GABA induces a transient rise in intracellular Ca²⁺.
It has been proposed that such induction may play a role in plasticity in Hermisenda. Here we report GABA-induced outward currents in photoreceptors of Hermisenda using
whole-cell patch-clamp technique. Individual photoreceptors were isolated as previously described. Inward Na⁺ and Ca²⁺ currents were minimized by substitution of Na⁺ with
epholine and using low external Ca²⁺ (1 mM). Outward K⁺ currents were blocked by
3 mM 4-aminopyridine and 100 mM tetraethylammonium. BAPTA (10 mM) was added to the
internal solution to block Ca²⁺. Under these conditions, inward or outward currents
were recorded. However, an outward current could be recorded upon the application of
10 μM GABA (figs.1 & b). Desensitization to GABA occurred after prolonged exposure.
Current was blocked by bicuculline (100 μM), a known blocker of GABA receptor. The
properties of the GABA mediated outward current was consistent with those of Ca²⁺ current:
(1) The current magnitude and the estimated reversal potential were altered
dependent on the external and internal CI concentrations. (2) The current was reduced by
D(GABA (100 μM), a known blocker of CI current. The inhibitory post synaptic potential at
the hair cell-photoreceptor synapses may be mediated by the CI current.

700.2
IDENTIFICATION AND CHARACTERIZATION OF PROTEIN TYROSINE PHOSPHATASES AT THE NEUROMUSCULAR JUNCTION. I. MEL TED K.L. HAGAN. Department of Neuroscience, HSUM, Johns Hopkins University
School of Medicine, Baltimore, MD 21205.
Protein tyrosine phosphorylation is prevalent throughout the nervous system
suggesting that it may be involved in the regulation of neuronal function. The
phosphorylation of proteins is regulated by the balance of protein kinases and
protein phosphatases. Although protein tyrosine kinases in the brain have been
demonstrated, much less is known about neuronal protein tyrosine phosphatases.
We have been using the neuromuscular junction as a model to study the role
of protein tyrosine phosphatases in the regulation of synaptic transmission and
plasticity. Twelve individual protein tyrosine phosphatases were isolated from
the rat skeletal muscle based on the consensus sequence of previously characterized
tyrosine phosphatases, including three novel ones. Previously we have shown that
PTP-1D, a cytoplasmic phosphatase containing two SH2 domains, appeared to be
abundant in the brain and muscle at the mRNA level and its phosphatase activity
was down-regulated by a four amino acid insert generated by RNA splicing. Using
affinity-purified antibodies, PTP-1D was identified as a 68-kDa protein most
abundant in the brain and heart, moderate in the skeletal muscle, lung, and liver,
least in the kidney. Its expression in the brain appeared to increase continually
during the development; from barely detectable in embryonic day 10 to maximal
on postnatal day 60. The catalytic domain of PTP-1D could be phosphorylated by
protein kinase C but not by CAMP-dependent kinase. Phosphoamino acid analyses
indicated that PTP-1D phosphorylation of PTP-1D was on serine residues. GST
fusion proteins containing SH2 domains of PTP-1D were made to study its interaction
with other proteins. The SH2 domains were found to bind to several
tyrosine-phosphorylated proteins in vitro, including nicotinic acetylcholine
receptors in the skeletal muscle. Studies are under way to investigate the effects
of PKC phosphorylation of PTP-1D on the phosphatase activity and to identify
the proteins that interact with PTP-1D.
ACTIVATION OF A CATIONIC CONDUCTANCE BY METABOTROPIC GLUTAMATERGIC AND MUSCARINIC AGONISTS IN CA3 CELLS IN HIPPOCAMAL SLICE CULTURES.
X.C. Gattas* et al., Brain Research Institute, University of Zurich, CH-8092 Zurich, Switzerland

An increase in a membrane cationic conductance in response to metabotropic glutamatergic or muscarinic agonists was characterized in CA3 pyramidal neurons using single-channel technique. Experimental conditions were chosen to block Na+ currents (1 mM TTX), Ca2+ currents (0.4 mM Cd2+), and K+ currents (internal Ca2+ instead of K+). Records were produced at room temperature.

In symmetrical HCl solutions, depolarizing pulses applied from a holding potential of -70 mV evoked inward currents between -40 and 0 mV (peak amplitude -400 - 600 pA at -20 mV) and became outward at positive command potentials (up to 100 mV), displaying a prominent Na+ reversal potential. Upon repolarization to -70 mV, I_Na slowly decayed producing a prominent inward tail current (v = 40 - 60 mV). At a given potential, the time constant of tail current decay was independent of prepulse potential. In contrast, when repolarization potentials were varied along the voltage axis (-120 to -60 mV) after a prepulse to 30 mV, deactivation kinetics of I_Na showed a strong voltage dependence, the decay time constant substantially increasing with depolarization.

In conclusion, experiments revealed that the channel producing I_Na was permeable to a number of monovalent cations including K+, Ca2+, Na+, and choline, but not to the anions Cl-. I_Na was reduced by TEA at high concentrations (15 mM), but not by 4-AP, and proved to be insensitive to cation channel blockers such as Cs+, amiloride, or gadolinium. Activation of I_Na did not require Ca2+-dependent or cytosolic Ca2+ rise.

Due to its slow deactivation kinetics, I_Na is likely to contribute to the sequence of afterpotentials following a depolarizing event in electrically active neurons.

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100.5 CYCLIC NUCLEOTIDE MODULATION OF THE HYPERPOLARIZATION ACTIVATED CURRENT, I_P, IN NIE-115 NEUROBLASTOMA CELLS.
C. Mathers* et al., Homemade, Hampton Marine Station of Stanford University, Pacific Grove, CA 93950

We recorded I_P in a neuronal cell line derived from mouse sympathetic ganglion. This inward and voltage-dependent current is activated at hyperpolarized potentials and tail currents measurements give a reversal potential of -30 mV. The instantaneous (I_0) and steady-state (I(V)) V curves are inwardly rectifying. The activation curve of I_P is fit by a single Boltzmann function with V = 70 ± 9 mV and the slope factor z = 3. Like I_K in cardiac and other cells, the inward current is inhibited by Cs but not by Ba2+. This channel distinguishes this inward rectifying K current. I_P amplitudes increase by about 144% when (Ca)_out is elevated and when Mn or Co are added. These results suggest that I_K is permeable to all divalent cations, including Ca, Mn and Co. Single channel currents have been measured with Ca as the carrier that have a similar I(V) curve and extrapolated reversal potential to I_P. The slope conductance is ~ 50 pS. In nystatin patch experiments, cAMP (1 mM 8-bromo cAMP) increases I_P by 400% (I_P0) and 151% (I_P1) at -95 mV. Cyclic GMP (1 mM 8-Br-cGMP) inhibited I_P by 36% (I_P2) and 46% (I_P3) at -95 mV. Cyclic AMP increases I_P amplitudes at all voltages, while the inhibition of I_P by cGMP occurs only at negative potentials. This cyclic nucleotide modulation of I_P may be the mechanism by which muscarinic receptor stimulation of muscarinic agonist application reduces I_P amplitudes by 35% at -90 mV. Muscarinic inhibition of I_P should decrease spike frequency and counteract the slow EPSP during synaptic transmission.

100.6 MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF AN INWARDLY RECTIFYING CONDUCTANCE IN RAT BRAIN.

A voltage sensitive inwardly rectifying chloride channel is present in hippocampal pyramidal neurons but not dentate gyrus neurons. This conductance has electrical properties similar to a chloride channel (designated CIC-2) that has been cloned and functionally characterized in a Xenopus oocyte expression system, and has been shown to have a significant role in modulation of neuronal responses to GABA mediated axons. Therefore, the CNS distribution of CIC-2 is of interest to understanding the neuron-specific mechanisms that can modulate GABA action. Using single cell hybridization methods to detect the CIC-2 mRNA, we have demonstrated that the CIC-2 mRNA is expressed to the same population of neurons in the hippocampus that contain a voltage sensitive inwardly rectifying Cl- conductance. In rat brain, expression of CIC-2 was restricted to neurons and choroid plexus but not detectable in glial cells. Although CIC-2 mRNA was widely expressed in cortical neurons, significant differences in signal intensity were observed regionally and in different cell layers. In subcortical structures CIC-2 expression was restricted to specific regions and neuronal cell types. These results suggest that in brain CIC-2 may have neuron specific functions in modulation of the chloride gradient that differ significantly from the proposed role in volume regulation of non-neuronal tissues.

In a recent study it has been shown that voltage-gated calcium currents, in pituitary cells, are flow sensitive (Ben-Tabou, Keller and Nussinovitch, J. Physiol. 476 1, 1994). This finding raised the possibility that local deformation of pituitary cells membranes can affect the voltage-gated calcium channels. We tested this possibility by exposing pituitary cells to solutions with different osmolalities, causing deformation of pituitary cell membranes by swelling or shrinking. In this study, pituitary cells were exposed to hypotonic solutions (165-200 mosmol) or hypertonic sucreose containing solutions (400-520 mosmol) by bath perfusion. The osmolality of the standard extracellular solution was 320 mosmol. Calcium currents were recorded with the whole-cell mode of the clamp-patch technique. Exposure to hypo-osmotic solutions resulted in two major effects: 1. Block of T-type calcium currents with no change in their voltage dependence of activation. 2. Negative shift (of about 10 mV) in the activation curve for high voltage activated (HVA) calcium currents. HVA calcium currents which were activated at negative test potentials (-50 mV to -10 mV) increased in size whereas those activated at more positive test potentials (+20 mV to +30 mV) decreased in size. Similar results were observed when the cells were exposed to hyper-osmotic solutions except that the block of HVA currents was not always associated with a shift in activation curve. These findings suggest that deformation of pituitary cell membrane by osmotic changes can regulate influx through voltage sensitive calcium channels.

Supported by the Israel Academy of Sciences and Humanities

701.3 NITRIC OXIDE SYNTASE (NOS) DOES NOT MODULATE CURRENT-DEPENDENT INACTIVATION OF N-Type CALCIUM CHANNELES BUT IT DOES SOMETHING TO ICa. J.J. Kenyon. Dept. of Physiol., Univ. of NL School of Med., Reno, NV 89557

I tested the hypothesis that Ca** entry via ICa, inhibited by stimulation of NOS and production of nitric oxide (NO), N-type ICa, was measured in chick dorsal root ganglion neurons using amphotericin perforated patches at 35±3°C. ICa,** was reduced by low [Ca**]i and 100 µM. Inactivation of ICa,** during 250 ms conditioning steps to +60 mV was monitored by 50 ms test steps (-8 mV). 10 s intervals separated the conditioning steps. As conditioning voltages were made less negative, test ICa,** decreased. When the conditioning voltage was at or below 0 mV, ICa,** could not be recovered. We propose that NO synthase does not play a role in the inactivation of N-type ICa,** but can modulate the inactivation of C-type ICa,**

701.5 PHARMACOLOGY OF LOW-THRESHOLD CALCIUM CURRENTS IN HIPPOCAMPAL CA3 PYRAMIDAL NEURONS. R.B. Avery* and D. Johnston. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77025

Hippocampal neurons possess multiple types of voltage-gated calcium channels (Rivera et al., J. Neurophysiol. 64:901). The contributions of specific channel types to cellular functions, however, is not well-established. Low-threshold calcium currents are thought to support complex firing patterns in CA3 pyramidal cells. Since the pharmacology of low-threshold calcium channels varies among neurons, we wish to screen potential blockers of these channels in CA3 cells.

Recordings were made from CA3 pyramidal neurons acutely isolated from rats 7 to 14 days old. Cel-attached patches confirmed the presence of low-threshold calcium channels on the soma of these cells. In 110 mM bath, single channel openings could be evoked from potentials as low as -60 mV. The slope conductance of single channels was less than 10 pS. Patches with several channels exhibited a rapidly inactivating macroscopic current. To screen putative blockers of these channels, we made whole-cell recordings in 5 mM calcium. From hypotonic and holding potentials, calcium currents activated at potentials more positive than -60 mV. We assayed low-threshold calcium currents with test pulses to -40 mV from a holding potential -50 mV. The activation at -40 mV was slowly activating and inactivating. The activation was about a third of the peak whole-cell current, which occurred between -10 and 0 mV. High-threshold currents were isolated by holding at +50 mV to inactivate low-threshold steps. Steps from -50 mV to 0 mV elicited a slightly inactivating current. The amplitude was about half that compared to currents evoked from a holding potential of -80 mV. 50 or 100 µM nickel strongly reduced the low-threshold component but only slightly blocked the high-threshold currents. (MH1473, MH14754, MHH4625, NS1153S)

701.6 REGULATION OF VOLTAGE-DEPENDENT CALCIUM CHANNELS OF MOUSE DRG NEURONS BY CHRONIC ELECTRICAL STIMULATION IN CULTURE. M.X. Li, M. Jia, B. D. Fields*, and P.G. Nelson. NICHD and NINDS, NIH, Bethesda, MD 20892

Previous research has shown that influx of calcium through voltage-sensitive calcium channels is responsible for the arrest of growth cone motility in mouse DRG neurons in culture. After chronic electrical stimulation, growth cones recover and become insensitive to repeated stimulation. (Biphasic stimulation was delivered at 6V in 0.5 s bursts at 10 Hz every 2 s for 40-70 h) One of the mechanisms of growth cone accommodation, whole cell voltage clamp experiments and calcium imaging using fura-2 were performed on mouse DRG neurons cultured in multi-compartmental chambers. The electrically-induced increase in calcium was significantly slower in the growth cones and cell bodies of DRG neurons after chronic stimulation (tau = 3 vs. 1.4s, p<0.001). High-threshold activated (IVA) whole-cell calcium currents were reduced in chronically stimulated neurons from 3.2 nA to 0.59 nA (p<0.02), measured at the end of a 200 ms stimulation pulse. Low-threshold activated (ILVA) were reduced from 0.74 nA to 0.095 nA (p<0.02). The membrane potential of the peak current was shifted from -15 mV in the control neurons to +9.1 mV (P<0.0002). The effective diameter of neurons in the control and stimulated groups. The HVA calcium currents inactivated more rapidly in the stimulated group. Our results suggest that development of the nervous system could be influenced by activity-dependent regulation of calcium conductance through VSCC, by ensuing effects on growth cone motility, and other calcium-dependent processes.
MORULATION OF RYANOINE RECEPTORS BY MITOHONDRIAL CALCIUM IN DORSAL ROOT GANGLION NEURONES. D. Bovet, P. Felix and R. Schlüter, Laboratoire de Physiologie Générale, Université Louis Pasteur, Strasbourg, France.

An extensive repertoire of ionic channels are modulated by changes in the concentration of cytosolic calcium ([Ca\(^{2+}\)]\textsubscript{i}) which, in many cases such as for ryanoine receptors, forms a bell-shaped dependence on channel activity. As yet, these observations have not been demonstrated in an intact neurone nor has the Ca\(^{2+}\)signalling pathway which modulates receptor function been identified. In this study, intact neurones were loaded with fura-2, we have attempted to identify the Ca\(^{2+}\)-signal which modulates ryanoine receptors using a variety of receptor agonists which elevate [Ca\(^{2+}\)]\textsubscript{i} through known pathways. The sensory neurones excitable, capsaicin (50nM-2uM) and 50nM KCI evoked biphasic elevations in [Ca\(^{2+}\)]\textsubscript{i}, consisting of a peak and plateau component. The peak Ca\(^{2+}\) rise was due to entry of extracellular Ca\(^{2+}\) which was attenuated and sustained at a plateau level for identification using 10uM FCCP to selectively inhibit Ca\(^{2+}\)-uptake into the organelles. The amplitude of the plateau was greater following application of capsacin than 50mM KCI. Activation of ryanoine receptors by 10nM caffeine elicited transient Ca\(^{2+}\) rises that were reversibly inhibited during the Ca\(^{2+}\)-plateau evoked by capsacin but unaffected by the plateau to 50mM KCI. Selective inhibition of the mitochondrial Na\(^{+}\)/Ca\(^{2+}\) exchanger with 10uM Gd3+ lowered the plateau amplitude observed with capsacin and restored the Ca\(^{2+}\) rises evoked by caffeine. These observations support a novel role of the mitochondrion as a long-term modulator of intracellular Ca\(^{2+}\) stores as well as a putative regulator of other Ca\(^{2+}\)-dependent events in the cytoplasm.


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CALCICUM INFLUX THROUGH P-TYPE CHANNELS ACTIVATES CLOSELY ASSOCIATED CALCIUM-DEPENDENT POTASSIUM CHANNELS AT THE MOUSE MOTOR NERVE TERMINALS, D.A. Protti and O.D. Uchate, Faculty of Medicine, University of Buenos Aires, Buenos Aires, Argentina.

Transmitter release is commonly shortened by the repolarization of the membrane, due to the onset of diverse potassium currents. At the mammalian motor nerve terminal, the main potassium conductances have been described as a voltage dependent K\textsuperscript{+} current, blocked by 3.4-diaminopimelic (3.4-DAP) and a calcium activated potassium current (IK(Ca)) sensitive to TEA and to Charybdoxin. We have previously reported that PTX and and A-IVA, two P-type calcium channel blockers, inhibit neuronal transmission, diminish quantal content and block calcium currents at the mouse motor nerve terminals (PNAS 89, 3333-3333, 1992; Neuroreport 5, 333-336, 1993).

We studied the activation of the IK(Ca) in the levator auris muscle-nerve preparation of the mouse by means of perineural recordings in the presence of 3.4-DAP (10mM). We have found that the IK(Ca) is abolished in the presence of 0.5mM EGTA (10mM) whole cells, two P-type calcium channel blockers, inhibit neuronal transmission, diminish quantal content and block calcium currents at the mouse motor nerve terminals (PNAS 89, 3333-3333, 1992; Neuroreport 5, 333-336, 1993).

We now report that a Ch\textsuperscript{2+} channel (p64), identical to that found in isolated ellipsoid cells, is present in the membrane of PF vesicles. The opening of this channel is associated with an increase in its state of phosphorylation and, in concentrations that also cause secretion, induced an increase in [Ca\(^{2+}\)]\textsubscript{i}. Secretion in response to 1mM Ca\(^{2+}\) was partially inhibited by the L-type channel blocker, nimodipine (NIM; 0.5 uM) and by tetraoda (TTX; 10 mU), which were combined. Secretion in response to TTX was unaffected by TTX, but abolished by NIM. Antagonism of secretion by TTX was reversed by the L-type channel agonist, Bay K8644 (10 uM). In contrast to NIM, neither the N-type channel blockers, nor the P-type channel blockers, D-agatoxin (0.1 uM), affected secretion. Vesicle acidification in response to TTX was partially inhibited by both NIM (0.5 uM) and D-agatoxin (0.1 uM), but was unaffected by D-agatoxin (0.1 uM). Vesicle acidification induced by TTX was abolished by NIM (0.5 uM), D-agatoxin (0.1 uM) and EGTA (10 mM), but not by TTX (10 uM). These data suggest that Ca\(^{2+}\) entry through voltage-gated Ca\(^{2+}\) channels is involved in coupling stimulation to both secretion and vesicle acidification; Ca\(^{2+}\) entry may contribute only to vesicle acidification. Supported by grants DK19743, NS07062 and NS12969.

THAPSGARGIN DISCHARGES INTRACELLULAR Ca\(^{2+}\) (Ca\(^{2+}\)\textsubscript{i}) STORES IN MOUSE CORTICAL ASTROCYTES. J.A. Edwards, H.H. Wolf and H.S. White, Anticonvulsant Drug Development Program, Department of Pharmacology and Toxicology, University of Utah, SLC, UT 84112.

Thapsigargin stimulates Ca\(^{2+}\) release from [Ca\(^{2+}\)]\textsubscript{i} stores by selectively inhibiting the Ca\(^{2+}\)-ATPase on the intracellular storage membrane. By poisoning the Ca\(^{2+}\) pump thapsigargin treatment produces a release of Ca\(^{2+}\) from the thapsigargin-sensitive Ca\(^{2+}\) stores. The present investigation was initiated to assess the [Ca\(^{2+}\)]\textsubscript{i} storage capacity of mouse cortical astrocytes. In an attempt to deplete [Ca\(^{2+}\)]\textsubscript{i} stores varying concentrations of thapsigargin (10, 30 & 100 nM) were high applied to a confluent monolayer of astrocytes for 10 minutes. Changes in the [Ca\(^{2+}\)]\textsubscript{i} of type-1 mouse cortical astrocytes (21-28 days in culture) were measured using an indo-1 based microfluorometry image system. Treatment with thapsigargin resulted in a dramatic increase (417, 646 & 6219 at 10, 30 & 100 nM, respectively) in the [Ca\(^{2+}\)]\textsubscript{i} over basal levels. This effect was only partially reversible upon subsequent wash-out with drug-free buffer. Of particular interest was the finding that the increase in [Ca\(^{2+}\)]\textsubscript{i} was highly dependent on the concentration of Ca\(^{2+}\)\textsubscript{i}. These results suggest that depletion of Ca\(^{2+}\)\textsubscript{i} stores was producing Ca\(^{2+}\) influx across the plasma membrane. Additional studies with dantrolene and ryanodine (to block [Ca\(^{2+}\)]\textsubscript{i} release) and nimodipine (to block voltage-sensitive Ca\(^{2+}\) channels) were unable to attenuate the thapsigargin-induced influx of Ca\(^{2+}\) and subsequent increase in [Ca\(^{2+}\)]\textsubscript{i}. These data suggest the hypothesis that the decrease in Ca\(^{2+}\)\textsubscript{i} stores may release some type of intracellular messenger (e.g., calcium influx factor) which would initiate refilling of Ca\(^{2+}\)\textsubscript{i} stores. (Supported by NIH grant 2 ROI NS-22200 and NIH/NIHDS contract NOI-NS-9-3228).

SINGLE CHANNEL BASIS OF THE MULTIPLE FUNCTIONAL COMPONENTS OF MACROSCOPIC L-TYPE Ca\(^{2+}\) CHANNEL CURRENT IN CLONAL PITUITARY CELLS. D. M. Paspa & E. S. Lefkath, Dept. of Neuroscience and Pharmacology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

In the presence of the dicyclotryptophanogen BAY K 8644 (1 uM), whole cell L-Channel Ba\(^{2+}\) currents in GEL cells consist of two functional components. These two components differ in their rate of deactivation, voltage dependence, sensitivity to BAY K 8644, and sensitivity to inhibition by the neuropeptide TRH. The simplest hypothesis which may account for these two components is one which invokes two populations of L-channels. To test this hypothesis, single channel recordings were obtained from cell-attached patches. To control the potential of the membrane outside the patch, the K\textsuperscript{+} ionophore valinomycin (10 uM) was applied to the cells. This treatment stabilized Vm at ~ -60 mV. In addition, rundown was much less pronounced (channel activity remained stable for hours) than when the typical high K\textsuperscript{+} external solution was used. Ensemble tail currents derived from recordings from multi-channel patches were fit by two exponentials, indicating that the two components were detectable in the presence of valinomycin. Two exponentials were also required to fit the ensemble tail current derived from one of the single-channel patches obtained thus far. The other single-channel patch had an ensemble tail fit well by one exponential. Obtaining more single channel patches will allow us to determine whether individual L-channels display the behaviors of one or both components of macroscopic L-current.

LEAD ATTENUATES L-TYPE CALCIUM CHANNEL INACTIVATION IN BOVINE CHROMAFFIN CELLS. L. Sun, J. B. Szuszkw, and N. Sperelakis, Dep. of Physiology and Biophysics, Univ. of Cincinnati, Cincinnati, OH 45267-0756.

Using whole-cell voltage clamp, we investigated the effects of inorganic lead (Pb\(^{2+}\)) on the voltage-gated calcium current recorded from bovine adrenal chromaffin cells. Currents were recorded by 200ms voltage step pulse to +10mV from a holding potential of -80mV. Extracellularly-applied Pb\(^{2+}\) (0.05, 0.1, 0.5, 1.0 uM) depressed calcium currents in a non-specific manner and acted as a potent and reversible blocker of Ca\(^{2+}\) channels, which is consistent with a competition by Pb\(^{2+}\) with Ca\(^{2+}\) at a binding site within the calcium channel. However, the contribution of lead to the suppression of Ca\(^{2+}\) current rundown. It has been indicated that the Ca\(^{2+}\)-dependent inactivation of L-type channels involves an activation of protein phosphatase and the channel dephosphorylation. These results suggest that Pb\(^{2+}\) may attenuate Ca\(^{2+}\) channel inactivation by maintaining Ca\(^{2+}\) channel in phosphorylated status. Supported by NIHES grants ES04090 and ES03656.
ACUTE EXPOSURE TO LOW LEVELS OF LEAD ALTERS CALCIUM CURRENT AMPLITUDES IN RAT PC-12 CELLS. C.C. Heg & V. Milete*. Dept. Comp. Biosci. & Environmental Toxicology Center, Univ. Wisconsin, Madison, WI 53706.

Lead is a known neurotoxin with varied and poorly understood mechanisms of action. We employed a whole-cell patch-clamp technique to examine the effects of low levels of lead on high-threshold voltage-dependent calcium currents in rat pheochromocytoma (PC-12) cells. PC-12 cells were placed in NGF-containing media for 4, 8, or 12 days followed by 24 h of recording. During recordings, the cells were first perfused for 5 minutes with lead-free solution, then switched to a 10 μM lead-containing solution for another 5 minutes, and finally washed again with lead-free solution. As previously reported, acute low-levels of lead caused a decrease in calcium current amplitude without an obvious change in activation kinetics in some cells (n = 7). However, unlike previous reports, acute exposure to the same low levels of lead also produced increases in calcium currents in other cells (n = 12). In both situations, the effect of lead was only partially reversible with washing. There was no systematic difference in the effects of lead among the 4, 8 or 12 day cultures. These data suggest complex interactions of lead with calcium channels in PC-12 cells. (Supported by NIH NS21278).

SELEcTIVE MODULATION OF T- AND L-TyPe CaLcium CuRRENTS BY STIMULATION OF m3 AND m4 MuScUlaRINe ReCePTORS SUBTYPES IN TRANSENCePTED 3T3 CELLS. S. V. Jones*, M. S. Rozengurt, and D. M. Rubin. Molecular Neuropharmacology, Departments of Psychiatry and Molecular Physiology and Biophysics, University of Vermont College of Medicine, Burlington, VT 05405.

Calcium currents were recorded from NIH 3T3 cells transfected with the m3 and m4 muscarinic receptor subtypes. Whole cell patch clamp recordings were made with (mM): NaCl 150, MgCl2 1, BaCl2 20, Heps 10 and Glucose 5. Patch pipettes (4-6MΩ) were filled with (mM) NMGC 120, MgCl2 4, EGTA 10, Heps 10, ATP 4, GTP 0.1, creatine phosphate 5, and creatine kinase 50 units/ml. Using a variety of stimulation protocols two types of voltage dependent calcium current were observed. A low voltage activated, transient, nifedipine-insensitive calcium current (T-type) and a slowly inactivating, high voltage activated calcium current that was dihydroprolyline sensitive (L-type). Simulation of cloned m4 receptors resulted in a pertussis toxin sensitive reduction in amplitude of the L-type calcium current but appeared to be without effect on the amplitude of the T-type calcium current. Conversely, activation of the m3 muscarinic receptor produced an increase in the amplitude of the T-type calcium current. The respective mechanisms of action of these muscarinic receptor subtypes will be addressed.

Noradrenergic potentiation of a low-voltage-activated calcium current in dissociated nucleus basalis neurons. S. Williams*, A. Pepeu, M. Mihlhehler and L. Beremh Dept of Physiology, CMU, 121I Geneva 4, Switzerland.

Nucleus basalis are neuronal clusters providing an important cholinergic input to the cerebral cortex and receive a prominent innervation from noradrenergic (NA) neurons of the locus coeruleus. Previous studies have indicated that noradrenergic neurons are endowed with the capacity to emit action potentials in bursts. These bursts are thought to be mediated by a potent low-voltage-activated (LVA) calcium current. We have studied the modulation of the LVA current in dissociated NB neurons using whole-cell voltage-clamp recordings. In the presence of TTX (0.5 μM), TEA (20 mM) and 4-AP (4 mM), NB neurons recorded with an internal solution containing (in mM) 130 Cs acetate, 20 CaCl2, 5 HEPES, 5 MgCl2, 0.1 BaCl2, 3 NaATP and 0.1 NaGTP, consistently displayed a transient LVA current. From a V0 of -90 mV, the LVA current was activated at potentials near -50 mV, had a peak amplitude (measured at -30 mV) of 223 ± 60 nA (n = 8, mean ± SD) and was reduced by amiloride (300 μM, n=3) or cadmium (200-400 μM, n=8). Perfusion of NA (50 μM) produced a long lasting (>15 min) increase in amplitude (44.3 ± 32.3% (+n = 4) in the peak amplitude of the LVA current. The addition of prazosin (1 μM), an α1 receptor antagonist, prevented this effect. Perfusing (40 μM) an α2 receptor agonist, mimicked the NA induced potentiation, producing an increase of 32.3 ± 23 % (n=7). These results suggest that NA, through the activation of an α2 receptor, can potentiate the LVA current and could therefore in certain conditions, favour the propensity of NB neurons to generate rhythmic bursts. (Canadian MRC and Swiss FN).

ACTIVATION OF GABAa AND GABb-ReCEPTOR MODULATORS DIFFERENT TYPES OF Ca2+ CURRENTS IN RAT NODULAR GANGLION NEURONS. J.I. Rushin* and H.C. Moises. Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109-0707.

Whole cell patch clamp recordings were obtained from nodose neurons acutely dissociated from 10-24 day old rats to characterize the Ca2+ channel types that are modulated by GABAa and GABb receptors. Components of high-threshold current were distinguished on the basis of their sensitivity to blockade by α,γ-GTGVIA, nifedipine, α,γ-AgaVA and α-CTX MVIC. Administration of the α,γ-AgaVA to nifedipine-resistant calcium current (EC50 = 0.3±0.1 μM) was blocked by cadmium (30 μM) suppressed high-threshold Ca2+ currents by 51.5±2.7% (n=5) and 20.7±2.4% (n=4), respectively. Blockade of the LVA type channels by α,γ-GTGVIA eliminated current suppression by cadmium in all cells tested (n=11). The inhibitory effect of DAGO was completely abolished by α,γ-GTGVIA in 12 of 30 neurons and partially reduced in the remaining 19 neurons. Administration of α-Aga IV in saturating concentrations failed to completely eliminate the μ-opioid sensitive component which persisted after blockade of N-type channels. These data suggest that α,γ-AgaVA-resistant component of the μ-opioid sensitive current was blocked by α-CTX MVIC in 9 neurons. The remaining neurons (n=9) contained an unidentified component of Ca2+ current that was resistant to blockade by α,γ-GTGVIA, dihydroprollyline, α,γ-AgaVA and α-CTX MVIC. This toxin-resistant component was reduced by DAGO. Dihydroprolyline-sensitive (L-type) current was unaffected by μ-opioid or GABb receptor activation. These data suggest that μ-opioid receptors in nodose ganglion neurons are negatively coupled to N, P, O and unidentified toxin-resistant Ca2+ channels, whereas GABb receptors are coupled only to N-type channels. (Supported by DA00365 to HCM).

SEROTONINERGIC MODULATION OF HVA CALCIUM CURRENTS IN DISOCIATED NUCLEUS BASALIS NEURONS. A. Pepeu, L. Beremh, M. Vescovi, and S. Williams. Dept. of Physiology, CMU, 121I Geneva 4, Switzerland.

A major source of cholinergic input to the cerebral cortex arises from nucleus basalis neurons (NBn). In addition to a low-voltage-activated Ca2+ current, NBn exhibit an important high-voltage-activated (HVA) Ca2+ current. The inhibition of the HVA current by baclofen and 5-HT was studied in guinea-pig acutely isolated NBn using the whole-cell voltage-clamp technique. The HVA current was isolated with TTX (0.5 μM), TEA (20 mM) and 4-AP (4 mM), and recorded with patch pipettes (in nM) 130 CsAc, 20 CaCl2, 5 HEPES, 5 MgCl2, 0.1 BaCl2, 3 NaATP and 0.1 NaGTP. A voltage step to 0 mV for 40 ms from a V0 of -90 mV activated a HVA current with a peak amplitude of 1.46 ± 0.47 (n=5). The HVA current could be distinguished pharmacologically into three components (L, P, and N). The total HVA current was reduced by 20 ± 4 % (n=4) with the application of picrotoxin (3-5 μM), an L-type blocker, by 30 ± 20 % (n=6) with o-pipecoloytoxin (10 μM), an N-type blocker, and by 35 ± 20 % (n=5) with α,γ-AgaVA (100 mM), a P-type blocker. The application of 5-HT (10 μM) or the 5-HT1a agonist 8-OHDPAT (10 μM) reduced the peak amplitude (at 0 mV) of HVA by 16 ± 13 % (n=5) and 20 ± 5 %, respectively. In the presence of picrotoxin (100 μM), 8-OHDPAT (10 μM) did not affect remaining HVA currents suggesting that 8-OHDPAT reduces only the N-type Ca2+ current. The GABAa agonist baclofen (0.1 μM) 10 % (n=5) reduction of HVA currents. We are presently determining which HVA current(s) is (are) modulated by baclofen. Taken together, these results suggest that, in NBn, the HVA Ca2+ current is composed mainly of type L, N and P. Serotonin and GABA may selectively modulate only one component of the HVA current. (Supported by Canadian MRC and Swiss FNS).
**701.10**

**SEBORRHON (5HT) MODULATES HVA CALCULUM CURRENTS IN RAT NECORTICAL PYRAMIDAL NEURONS.** Feoktistov, R. C.* Depts. of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38163.

Necortical pyramidal cells were acutely dissociated from the sensorimotor cortex of rats (P7 or adult) and whole cell voltage-clamp recordings were made. SHT induced a decrease in HVA currents in a reversible and dose-dependent manner. In a given, 10-40% of the total current was modulated by SHT, with no change in voltage-dependence. The SHT effect was mimicked by the SHT agonist 5-CT and by the SHT1 agonist Nov-202PHAT. The SHT effect was blocked by the SHT1 antagonist AM 1721, but not by the SHT2 receptor agonist or antagonist. The data suggest that the modulation is mediated by SHT1A receptors. Under our standard internal Ca2+ chelation conditions (10 mM EGTA or 20 mM BAPTA), the modulation was unaffected by 5 μM nigfopoly and partially occluded by saturating doses of α-CgtX GIVA (1 μM). The combination of these two agents completely blocked the modulation, suggesting that N- and P-type channels were modulated. The modulation of N- and P-type current by SHT was rapid in onset (of several hundred ms) and partially reversed by preloads to <120 mV. In the presence of GTPβs, the modulation was irreversible. These data indicate the involvement of a 5-HT receptor in the modulation and suggest utilization of a membrane-delimited pathway. When the initial solution contained 0.1 mM BAPTA as the only chelator, L-type currents were reduced in addition to N- and P-type currents. This modulation appeared slower in onset than that of N- and P-type currents. Under all chelation conditions, SHT reduced the slow tail currents generated by L-Type channels in the presence of Bay K 8644. Supported by NINDS grant #S399527180. α-Agt-X IVA was a gift from Pfizer, Inc.

EXCITATORY AMINO ACIDS: PHARMACOLOGY

**701.2**

**KETAMINE-INDUCED ALTERATION OF NITRIC OXIDE SYNTHETASE ACTIVITY IN RAT CAUDATE NUCLEI.** R.A. Mueller, M.D., Ph.D.* and R.D. Blunt, B.S., Departments of Anesthesiology and Pharmacology, UNC-Chapel Hill, Chapel Hill, NC 27599.

It has previously been reported that pretreatment with inhibitors of nitric oxide synthase (Type 1) (NOS) can antagonize the anesthetic effects of ketamine in rats, but the effect may be due to decreased delivery of ketamine to its site of action. In the present study in *vivo* NOS synthase activity was used to assess caudate NOS during the onset, maintenance and disappearance of ketamine anesthesia.

Rats (60-120 g) given saline (2 ml/kg) or ketamine (75 mg/2 ml/kg) i.m., at zero time were used to study NOS activity in 15 min (onset) or 60 (maintenance) minutes after IM saline or ketamine. Each rat received 5-10 μg of i3H-arginine i.v. and as killed by decapitation 5 minutes later, with the immediate collection of plasma, liver, parietal cortex, cerebellum, and caudate nuclei samples.

3H-citrulline was measured by ion exchange and HPLC with all tissue values corrected for contamination of the tissue by plasma 3H-citrulline calculated to be within the tissue.

Synthesis of caudate 3H-citrulline was increased 50% in rats that received ketamine 15 minutes previously (p < 0.05), but not at 30 or 60 minutes. No significant changes were noted in the cerebellum or parietal cortex.

These results suggest that after ketamine, the NOS activity is briefly increased acutely but returns to normal even though anaesthesia persists. NOS activity changes do not parallel the time course of anesthetic effects.

**701.3**


The actions of dopamine (DA) agonists in the nucleus accumbens (Acb) are thought to be translated into locomotor activation through decreases in ventral pallidal GABA. Blockade of glutamate function in the Acb by antagonism of glutamate antagonists such as MK-801 also induces locomotor output. Some have suggested that MK-801 produces locomotor stimulation through mesolimbic DA, although support for this proposition is equivocal. For example, we have previously failed to find significant increases in Acb DA overflow by pharmacologically relevant doses of MK-801. This apparent behavioral paradox provides support for a role of Acb DA in the stimulatory effects of this glutamate antagonist. DA depletion lesions of the Acb significantly attenuated the locomotor-activating effects of amphetamine applied focally to this structure while the stimulatory actions of MK-801 were not altered. The second series of studies was directed at evaluating the role of the pallidal GABA in mediating the motoric actions of DA agonists and MK-801. Using *in vivo* microdialysis procedures, amphetamine was found to decrease pallidal GABA while MK-801 failed to have an effect despite producing pronounced increases in locomotor activity. Injection of muscimol (100 nmol) into the ventral pallidum attenuated the locomotor-activating effects of amphetamine but not MK-801. The locomotor activating effects of MK-801 do not appear to involve Acb DA nor pallidal GABA.
702.5
7-Cl-Kynurenic acid (7-Cl-KYN), a selective and potent antagonist of the glycine co-agonistic site of the NMDA receptor, can be produced from L-4-Cl-kynurenic acid (L-4-Cl-KYR) by the action of quinolinic acid peptidase, a gliarial enzyme which catalyzes the biosynthesis of kynurenic acid from L-kynurenine (4). The mechanism of action of 4-Cl-KYN is not yet known. Intrastriatal injection of 120 nmol quinolinic acid (QUIN) into the striatum (over 2 hrs) resulted in a 45 ± 3% decrease in GAD activity. The production of 7-Cl-KYN from 4-Cl-KYR was verified by HPLC analysis of brain extracts. 181 ± 13 nmol/kg of 7-Cl-KYN were recovered from a normal striatum infused with 135 nmol 4-Cl-KYR for 4 hrs (N=12). The production of 7-Cl-KYN was decreased by 51% after intrastriatal injection of fluoro-2:1-KYN (1 mol/L µl, i. str.; N=13), but was increased by 204% in the astrogliotic striatum, i.e. seven days after an intrastriatal QUIN (360 nmol) injection. These data indicate that astrocytes can produce neuroprotective quantities of 7-Cl-KYN from 4-Cl-KYR in the striatum in vivo. Supported by a grant from the Huntington's Disease Society of America (to P.G.).

702.7
LEARNING IMPAIRMENT INDUCED BY CHRONIC INFUSION OF QUINOLINIC ACID - PROTECTION BY MEMANTINE. W. Danysz, M. Misztal, R. K. Filipkowski, L. Kaczmarek, and J. Stangier-Kramska. Dept. Pharmacol., Merz+Co, 60318 Frankfurt /Main, Germany, Nencki Institute, 02-093 Warsaw, Poland.
We have recently demonstrated that chronic treatment with memantine resulted in a decrease in the density of muscarinic receptors at sites of the hippocampus, pons, and thalamus. Parallel infusion of a competitive NMDA antagonist memantine (1-amino-3,5-dimethyl-ladamantane), 20 mg/kg/day by another minipump prevented the learning deterioration induced by choline. The treatment with memantine resulted in steady-state plasma levels of 1.2 µM which should assure inhibition of NMDA receptors and is similar to levels seen in demented patients treated with this agent. Hence, if the glutamate hypothesis of dementia is accepted the neuroprotective activity of memantine in SDAT patients might be expected.

702.9
NMDA, BUT NOT AMPA, BLOCKADE REDUCES MONOSODIUM GLUTAMATE (MSG) INDUCED DAMAGE IN NEONATAL RATS. B. Milburn, J. Parkes, T. Bambrock, R. Telford, D. Bannerman and W. Sagard. Neuroscience Research Unit, Nipissing University, 100 College Dr., North Bay, Ontario, Canada, P1B 8L7.
In two experiments, we investigated whether NMDA and/or AMPA receptor blockade mitigates the physiological and behavioural damage associated with neonatal MSG injections in the rat. In the first experiment, 10 day rat pups received two injections on postnatal days 2, 3, 5, 7 and 9. The first injection was either saline (SAL) or MES-801 (0.1 mg/kg; sc) while the second injection was either saline or MSG (4 g/kg; sc). Two groups received 3 treatments: MES-801/SAL and MES-801/MSG. In the second experiment, we tested the protective effect of MES-801 and/or NBQX injection prior to MSG administration. Behavioural testing revealed the expected SAL/MSG induced learning deficit in the water maze test. The tail flick test in postmortem analyses revealed organ weight reductions in MSG treated rats. We conclude that MB-801, but not NBQX, protects against damage associated with neonatal MSG treatment. ACKNOWLEDGEMENT: The authors thank NOVO NORDISK for their contribution of NBQX.

702.10
The aim of the present study was to characterize the pharmacological mechanisms involved in the central muscle relaxant action of flupirtine, a novel non-opioid centrally acting analgesic agent. For this purpose in urethane-anesthetized rats, the action of flupirtine was investigated on the mono-synaptic Hoffmann-(H) reflex recorded from plantar foot muscles and on polysynaptic flexor reflexes recorded from tibialis muscle. Intraperitoneal (1-10 mg/kg) and intrathecal (0-330 nmol) administration of flupirtine depressed polysynaptic flexor reflexes in a dose-dependent manner without affecting monosynaptic H-reflexes. The effect of intrathecal injection of flupirtine was prevented by co-administration of the mixed alpha1-adrenergic antagonist yohimbine (10 nmol) and the excitatory amino acid antagonist and N-methyl-D-aspartate (NMDA; 10 nmol), but neither by co-administration of the alpha2 adrenergic antagonist prazosin (10 nmol), the GABA-A antagonist bicuculline (1 mmol), the GABA-A antagonist phaclofen (100 nmol) and the non-NMDA agonist amino-3-hydroxy-5-carboxylate (AHPA; 0.1 pmol) nor by pre-treatment with the benzodiazepine antagonist flumazenil (5 mg/kg). These observations suggest that alpah2 and NMDA receptors are involved in the mediation of the muscle relaxant activity of flupirtine.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
**EFFECTS OF MK 801 AND FLUPIRTINE ON ISCHEMIC RETINAL DYSFUNCTION.** F. Block1, M. Schwarz1, G. Pergande2, S.W. Schäfer1, 1 Dept of Neurology, RWTH, 52057 Aachen, Germany; 2 ASTA Medica, D-66421 Ludwigshafen, Germany.

Electrophysiological data suggest that flupirtine exerts a muscle relaxant effect via antagonistic action at the NMDA receptor (Schwarz et al, present meeting). Transient occlusion of both common carotid arteries (24 minutes) in normotensive rats induces retinal ischemia as reflected by a reduction in amplitude of the b-wave of the electroretinogram. Anesthesia was induced with ketamine (124 mg/kg, i.p., 1992). In the present study the effect of flupirtine on the reduction in amplitude during ischemia and on the recovery during reperfusion of the b-wave was compared to that of the NMDA antagonist MK 801. The electroretinogram was recorded from pentobarbital-anesthetized rats before, during and after transient occlusion of both common carotid arteries. Data were analyzed in a modified two-way ANOVA (P<0.05). In preliminary experiments ketamine (10 mg/kg, s.c.) protected the retina from ischemic damage. Flupirtine (1 mg/kg, s.c.) decreased the protective effect of ketamine (10 mg/kg, s.c.) (Student's t-test, P<0.05).

**WITHDRAWN**

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**AN OPEN PROBABILITY ESTIMATE FOR CNS GABA\(_A\) CHANNELS.** M.V. Jones* and G.L. Westbrook. The Vollum Institute for Advanced Biomedical Research, Portland, OR 97201.

Inhibitory synaptic transmission in the CNS is shaped, in part, by the probability (\(P_o\)) that postsynaptic GABA\(_A\) receptor channels will open once they have bound GABA. In the past, \(P_o\) has been estimated by examining the proportion of time spent open during long bursts in steady-state single channel records. (Newland et al., 1991). At synapses, however, the GABA concentration is set at steady state. Disaggregation of GABA and receptor desensitization may play a role in determining time-dependent changes in \(P_o\) that shape the synaptic current. To assess \(P_o\), after pulses of 4 FRABA, we examined the variance of GABA-activated currents (by the method of Sigworth, 1980). Outside-out patches from cultured postnatal rat hippocampal neurons were voltage-clamped, and currents were activated by rapid applications of a saturating GABA concentration (100mM, 1 to 100ms duration, close solution exchange time, 22°C). Sampled currents (I=20kHz, 100/point) activated rapidly (2-3ms, 10-90%), showed multiphasic desensitization (\(t_m=20ms\) and \(t_d=1ms\)) and multiphasic deactivation (\(t_m=10, 50\) and \(200ms\)). For a series of up to 100 currents in a patch, the variance of each sampled data point about its mean was calculated using local averaging of every three currents to compensate for rundown. Fits of variance (\(\sigma^2\)) vs mean (\(\mu\)) were fitted to \(\sigma^2=\alpha\mu+\beta\), where \(\alpha\) and \(\beta\) are independent constants and \(\mu\) is the estimated apparent conductance, \(\mu\), but not \(N\), varied with potential (0.74±0.1pA/nS, 1.5±0.3 (N=4, 2.4±0.5 (N=5) at -30 to -40 and -90mV), giving a slope conductance of 30pS, close to the 26±2pS main conductance obtained by direct single channel measurements from these neurons. The mean peak current divided by the maximum current possible (\(I_{peak}/I_{max}\)) gave a mean \(P_o\) between 0.56 and 0.67, independent of potential. Our estimate of \(P_o\) at the peak of responses to saturating pulses of GABA is ~25% lower than that found within long bursts of steady-state single channel activity (Newland et al., 1991). These results predict that inhibitory postsynaptic currents in the CNS represent an opening of not more than ~40% of the channels that are present opposite the sites of GABA release.

Supported by NIBH grants T52-DA07282 (M.V.J.) and NS25494.

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**GABA RECEPTORS: FUNCTION III**

**ELECTROPHYSIOLOGICAL CHARACTERIZATION OF RECOMBINANT GABAR ISOFORMS COMPRISING THE MAIN CEREBELLAR GABAR EXPRESSED IN L929 MOUSE FIBROBLAST CELLS.** N.C. Saxena* and R.L. Macdonald. Dept of Neurology, Univ of Michigan, Ann Arbor, MI 48104.

Recent immunoprecipitation studies using mixtures of subunit-specific antibodies have provided valuable evidence for the subunit composition of GABAR isoforms in the rat cerebellum (C.J. Ragan et al. 1993. Biochem. Soc. Trans., 21:622-626). Based on their findings, these main GABAR isoforms comprise approximately 90% of total rat cerebellar GABARs. These are \(\alpha_6\), \(\beta_2\), or \(\delta\) subunits. Transfections with all three subunit combinations yielded a high percentage of GABA-responsive cells suggesting that GABAR isoforms predicted by immunoprecipitation studies of Ragan et al do indeed constitute functional GABARs in these cells. The concentration-response (CR) curves for \(\alpha_6\), \(\beta_2\), \(\delta\) and \(\delta\) GABAR isoforms indicate that the substitution of the \(\alpha_6\) subunit for the \(\alpha_6\) subunit and the substitution of the \(\beta_2\) subunit for the \(\beta_2\) subunit decreases the EC50 values for GABAR isoforms. \(\alpha_6\), \(\beta_2\), whole-cell currents were enhanced by disacchar so that the current is decreased in the presence of low concentrations of ketamine. The three different GABAR isoforms displayed differences in their response to disacchar so that the current is decreased in the presence of low concentrations of ketamine. The three different GABAR isoforms displayed differences in their response to disacchar so that the current is decreased in the presence of low concentrations of ketamine. Different channel characteristics of these GABAR isoform are under investigation.
703.3
γ-AMINOBUTYRIC ACID INCREASES INTRACELLULAR CALCIUM CONCENTRATION IN CULTURED RAT CORTICAL NEURONS.
Recent reports suggest that a major inhibitory neurotransmitter, γ-aminobutyric acid (GABA) increases intracellular calcium concentration ([Ca²⁺]ᵢ) in neonatal hippocampal neurons. In this study, we examined GABA-induced Ca²⁺ mobilization in cultured rat cortical neurons by using calcium indicator, fura-2/AM. Frontal cortex was isolated from Wistar rat pups at embryonic day 18 and cultured for 5-6 days. [Ca²⁺]ᵢ in single cells was measured with fluorescence microscopy/video camera systems. GABA and GABA-A receptor agonist, applied for 50 sec transiently, increased [Ca²⁺]ᵢ dose-dependently. The increase was inhibited by GABA-A antagonists, bicuculline and picrotoxin, but not by GABA-A antagonist, phaclofen. The [Ca²⁺]ᵢ increase was inhibited by EGTA and a Ca²⁺ channel blocker, nifedipine, the [Ca²⁺]ᵢ increase could be explained by Ca²⁺ influx through voltage-gated Ca²⁺ channel. These results suggest that GABA increases [Ca²⁺]ᵢ chiefly through GABA-A receptor and voltage-gated Ca²⁺ channel in immature cortical neurons.

703.5
It is known that activation of GABA-A receptors in LHRH secreting GTI-7 neurons results in membrane depolarization and calcium entry via voltage dependent calcium channels resulting in a calcium signal. We have analyzed this calcium signal using fura-2 based calcium imaging and the perforated patch-clamp technique. Instantaneous replacement of chloride ions in the medium with isotonic, resulted in a significant increase in the muscimol-induced calcium response. This increase, however, was transient and after 15 min of perfusion in zero chloride medium, the signal amplitude was only 50% of the normal chloride condition. Replacement of chloride with gluconate dramatically increased the calcium response since gluconate chelates extracellular calcium ions. Under current clamp, muscimol application induced a depolarizing response of 26 ± 10.0 mV with a few HTX-sensitive action potentials. In the absence of external chloride, the amplitude of muscimol-induced depolarization was smaller but the frequency of spikes was increased. Muscimol-induced depolarization and calcium increase were abolished in cells treated with furosemide (0.1mM) for 20 min. Ethacrynic acid (0.3mM) also reduced the calcium signal by 70%. Depletion of intracellular calcium stores by thapsigargin (100 nM) decreased the calcium response by 60% suggesting that release from stores contributes to the GABA-A mediated cellular calcium response. The data demonstrate that a cooperative action of furosemide- and ethacrynic acid-sensitive CI-pumps maintain outwardly directed driving force for chloride ions.

703.7
CHARACTERIZATION OF GABA, AND GABA-RECEPTORS IN TURTLE RETINAL GANGLION CELLS. Y. Liu and E.M. Lastennet
Moran Eye Center, Univ. of Utah, Salt Lake City, UT 84132.
We recently reported that turtle retinal ganglion cells express both GABA, and GABA receptors coupled to Cl channels. Experiments were carried out to further characterize the kinetic and pharmacological properties of these channels. Standard whole-cell patch recordings were made from isolated, cultured turtle retinal ganglion cells. Test agents were applied by a superfusion system. Application of GABA (1-100μM) induced a prominent inward current with two components in all ganglion cells tested. The decay of the GABA response could be fitted with a single exponential time course plus a steady state. The time constant of the transient current is about 3sec, while the steady state current is about 1000sec. GABA(1-100μM) activated a sustained current, which is very similar to the steady state current induced by GABA. Bicuculline (50-100μM) inhibited the transient but not the sustained current induced by GABA. Porcine (Ptx, 100μM) totally blocked both components of GABA response. Surprisingly, when Ptx was washed out, subsequent application of GABA only activated the sustained current. It took another application of GABA to induce both components. To further test if Ptx blocks the two types of GABA receptors via different mechanisms, lower concentration of GABA (1-10μM) was applied twice (interval, 2min). It was found that the second application of Ptx inhibited more transient current as compared to the first application of Ptx. This is consistent with the mechanism of open channel block. On the other hand, the percentage of the sustained current inhibited by Ptx is constant regardless of how many times Ptx was applied, which indicates that the inhibition of the sustained GABA response is mediated by some other mechanisms then open channel block. Our data suggest that the GABA and GABAB receptors in turtle retinal ganglion cells are coupled to Cl channels with different kinetic and pharmacological properties. Moreover, we first report that Ptx may regulate the GABA, and GABAB receptors via different mechanisms.

Supported by NH grant EY 00772 and Research to Prevent Blindness, Inc.

703.4
We have tested the sub-population of retinal neurons in the II organ of the crayfish with identified by using a monoclonal anti-Leu-enk antibody (Neurom). Only eight cell bodies with diameters between 35 to 50 μ located in the most superficial layer of the II organ were immunopositive. These cells may be the same found to react to an antibody against the crustacean hyperactive hormone (Van Herp & V. Buggenum, Experimenta 35: 1527, 1979). The isolated II organ cells were kept in culture several days. GABA was applied in microdrops onto different regions of the neuron. A dose-dependent depolarization was elicited, resulting in the firing of action potentials. This effect was reversibly blocked by Picrotoxin and Bicuculline and was mimicked by Muscimol. Under voltage-clamp conditions, the reversal potential for the GABA-induced current was shifted to more positive values. This suggests that chloride mediates the effect of GABA on X organ cells.

This work was supported by CONACyT grant number 1402-N9206

703.6
GABA-INDUCED GABA RELEASE IN CULTURED EMBRYONIC RAT THALAMIC NEURONS IS Ca²⁺-DEPENDENT AND TTX-INSENSITIVE. O.Y. Lin*, J. Vauthrin, A. Schaffner, K.M. Tang, W. Ma and J.L. Barker. Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.
In embryonic and early postnatal stages, GABA has excitatory effects on most if not all neurons expressing functional GABA-A receptor-coupled Ca channels. Due to a depolarizing CI gradient, activation of GABA-A receptor-coupled Ca channels depolarizes neurones, which in turn may activate voltage-dependent Ca²⁺ channels and lead to increases in cytosolic Ca²⁺ (Ca²⁺;). But in some cells GABA induced increase in Ca²⁺ does not appear to be mediated by classical CI channel coupled GABA-A receptors. In culture embryonic (E17) rat thalamic neurones, we found that 10μM GABA induced or increased the frequency of miniature synaptic-like transients (minis) superimposed on persistent CI current response. The minis reversed polarity at E₅₀, were blocked by bicuculline and decayed with kinetics similar to GABA-activated CI channels suggesting they were GABAergic. The agonist concentration that induced minis was lower than that required to activate CI channels. Interestingly, the GABA-A receptor/CI channel agonist muscimol readily induced CI current response but failed to mimic GABA in triggering minis. GABA-induced GABA release was dependent on extracellular Ca²⁺ and insensitive to 2μM TTX. We conclude that GABA can trigger GABA transients in cultured embryonic rat thalamic neurones through an action of GABA that does not involve classical GABA-A receptor/CI channels.

703.8
The GABA-A agonist baclofen has been shown to suppress synaptic transmission in subregions of the hippocampus and in the perifrom (olfactory) cortex. Here we report a laminar selectivity of suppression of synaptic potentials in the olfactory cortex. In brain slice preparations (n=27), baclofen suppresses extracellularly recorded field potentials at the intrinsic fiber synapses proximal to the superficial pyramidal cell bodies (layer Ib) while leaving the afferent fiber synaptic potentials recorded at the distal dendrites (layer Ia) little affected. The suppression of synaptic potentials was significantly stronger in layer Ib (n=5) than in layer Ia (n=3) at each of the following concentrations: 0.1, 1.0, 10, 100 and 500 μM. At the concentration of 100 μM, suppression in layer Ia was 5.1 ± 6.3% (n=5); suppression in layer Ib was 68.3 ± 6.7% (n=5). Suppression of intrinsic fiber synaptic transmission in layer Ib had an E₅₀ = 92.5 μM. This dose dependent selective suppression of intrinsic fiber synaptic transmission is also correlated with an increase of pameide-pulse facilitation. These results suggest that afferent and intrinsic synaptic inputs may be differentially modulated by the activation of GABA-A receptors and that this selective suppression is at least partially mediated via a pre-synaptic mechanism.
GABA RECEPTORS: FUNCTION III

703.0 DEPOLARIZING AND HYPERPOLARIZING GABA RESPONSES IN THE XENOPUS SPINAL CORD ARE MEDIATED BY GABA A RECEPTORS.
I. Robbough and N. Spatz* Department of Biology, UCCS. La Jolla, CA 92038.

703.11 GABA ACTIVATES TWO DISTINCT GABA A POSTSYNNAPTIC RESPONSES IN CA1 PYRAMIDAL CELLS OF RAT HIPPOCAMPAL SLICES. T.M. Flamm* and J.C. Lacaille, Center for Research in Neurological Sciences and Department of Physiology, University of Montreal, Montreal, QC, Canada H3C 3J7.

703.12 THE INHIBITION OF PRIMATE SPOINOTHALAMIC TRACT NEURONS PRODUCED BY STIMULATION IN PERIQUADRANTAL AREA IS REDUCED BY SPINAL BICUCULLINE. P. Lim* V.B. Penc and W.D. Willis, Department of Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, TX 77555-0843.


GABA RECEPTORS: FUNCTION III

T03.15 EVALUATION OF FLUORESCENCE AND ELECTROCHEMICAL DETECTION OF G-AMINOBUTYRIC ACID IN MICRODIALYSIS SAMPLES


A technique of in vivo microdialysis has been used in number of studies to characterize extracellular release and uptake of g-aminobutyric acid (GABA) in the brain. It was shown that at least 50% of GABA overflow in the striatum of freely moving rats is of neuronal origin. Determination of GABA requires an ultrasensitive analytical method, since GABA levels in typical microdialysis sample are often in the range of 10-5 pmol (50 nmol). Previously we developed an isotropic HPLC method based on electrochemical (EC) detection of GABA derivatized with OPA-butylthiol. The limit of detection was 50 fmol GABA. However, the excess of thiol which is oxidized on the electrode and a high acetone (50%) in the mobile phase are most probable causes of shortened life-time of the electrode, gradually increasing background signal and noise levels. The result is that a substantial portion of "research time" is spent on system maintenance.

Fluorescence detection of OPA derivatized amino acids is a more common way to determine physiological amino acid in body fluids. Normally, by using gradient elution and OPA/mercaptoethanol derivatization the limit of detection for GABA is only 0.5 pmol. To achieve GABA detection of 50 fmol we developed a new method based on microbore columns and optimized isotropic separation with a step gradient. The step gradient is achieved by a low pressure switching valve installed at the pump inlet. The whole system is fully automated allowing analysis of up to 80 samples/day.

Correlation between these two techniques was studied for microdialysis samples from the rat. Advantages and limitations of both methods are discussed. Ref. 1. Kehr J. and Ungerstedt U., J. Neurochem. (1986) 51, 1308.

T03.17 HETEROREGONETY OF ALLOSTERIC COUPLING IN GABA A RECEPTORS REVEALED BY A SUBUNIT SPECIFIC ANTIBODIES. K.H. Huht, S. Endo and R.W. Olsen, Department of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024.

GABA receptors are composed of multiple binding domains which are allosterically coupled with each other in a functionally relevant manner. Differential degrees of coupling observed in different brain regions have been interpreted as the existence of receptors with diverse subunit combinations.

In the present study, the subunit specificity of allosteric interaction between GABA and benzodiazepine binding sites were studied using a subunit specific antibodies. In the TX-100 soluble extract from rat whole brain, GABA A receptors immunoprecipitated with a1 antibodies had a significantly lower maximal enhancement of [3H]flunitrazepam binding by GABA than those immunoprecipitated with a2 or a3 antibodies (35 +/- 2% for a1, 75 +/- 4% for a2 and 83 +/- 1% for a3). On the other hand, receptors containing a1 or a3 subunits have higher affinities for the benzodiazepine (-2 x 10 -10 M) and 36 +/- 20 nM, respectively) as opposed to those containing a2 subunits (142 +/- 27 nM). These differential coupling efficiencies endow unique functional and structural properties to different a subunit containing receptors. In addition to the previously identified benzodiazepine pharmacology, it would be of interest to see if this heterogeneous binding profile can also be translated into the differential coupling with the channel gating domain.

Supported by NIH grant NS 28772.


Previous studies detected high affinity opioid binding sites in synaptosomal plasma membrane (SPM) and microsomal (MI) fractions of rat brain. Since they were found to be uncoupled from G-proteins in the latter, they were postulated to be desensitized sites. To explore this hypothesis, female Wistar rats were s.c. injected by increasing doses of morphine twice daily for 5 days. Western-blotting with specific antiserum, pertussis toxin catalysed ADP-ribosylation and photoaffinity labeling studies all showed that prolonged exposure to morphine elevated the amount of G-proteins which was calculated 63% and 30% in SPM resp. MI in [35S]GTPyS binding studies. The Bmax for the μ-opioid agonist [3H]DAMGO nearly doubled in MI after morphine treatment. These up-regulated sites displayed profound sensitivity to GTP unlike the μ-sites detected in MI of untreated rats.

These results suggest that regulation of G-proteins represent part of the molecular changes that underlie morphine effects in brain, and further emphasize the significance of intracellular events.

Supported by OKTA-8359 research grant.


The steady-state levels of MOR mRNA were measured by solution hybridization in samples microdissected from rodent brain and spinal cord. In adult male rats, a 7 day treatment with morphine (2 X 75 mg pellets, implanted sc on days 1 and 4), which produces a 11-fold shift in morphine dose response curves, increased the levels of MOR mRNA in the dorsal horn of spinal cord (SpC), nucleus raphe magnus (NRM), periaqueductal gray (PAG), medial thalamus (Thal), hypothalamus (Hyp) or somatosensory cortex (Ctx) of treated rats as compared to placebo controls. The MOR mRNA levels ranged from 0.7 pg/μg RNA in Ctx to 15.0 pg/μg RNA in Thal. The CBXK (μ-deficient) mice were found to be 7-fold less sensitive to systemic morphine than CD-1 mice, as assessed using the tail flick test (ED50 estimates were 29.4 and 4.2 mg/kg sc, respectively). CBXK mice were also found to have lower MOR mRNA levels than CD-1's in SpC (by 28%), NRM (by 43%), PAG (by 35%), Hyp (by 49%) and Thal (by 15%), but not in Ctx. The CD-1 MOR mRNA levels ranged from 0.04 pg equiv./μg RNA in Ctx to 0.52 pg equiv./μg RNA in Thal. Thus, while no change in MOR mRNA occurs in morphine tolerant rats, the low levels in CBXK mice may result in fewer opioid binding sites and contribute to the functional difference between CD-1 and CBXK strains. Supported by DA07274, DA01457, DA00196 and VZ Research Foundation.

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The steady-state levels of OR mRNA were measured in male C57-B16 mice rendered tolerant to the antinociceptive effects of the selective DOR opioid [D-Ala²-Nal]enkephalin II (DOR II) and morphine (MOR). After 7 days of administration of MOR (10 mg/kg, s.c.) or DOR II (10 mg/kg, s.c.) for 4 days, the OR values for MOR and DOR II were increased 5- and 8-fold, respectively, relative to saline controls. DOR mRNA levels were measured by solution hybridization in samples obtained by microdissection of CNS regions of tolerant and control mice. DOR transcript levels, in pg/ug of total RNA, were highest in the caudateputamen (0.47) and frontal cortex (0.32). Moderate levels were observed in n. accumbens (0.26), olfactory cortex (0.20), PAG (0.2), and hippocampus (0.14). The medial thalamus (0.03) and cerebellum (0.04) displayed relatively low levels of DOR mRNA. The OR mRNA levels in the CNS regions of MOR or DOR II tolerant mice did not differ significantly from controls in any CNS region studied. These data suggest that altered levels of OR mRNA are not one of the adaptive changes observed in mice tolerant to the antinociceptive effects of MOR or DOR II. Supported by DA07274, DA01457, DA00198, the NIMH Research Foundation and a grant from the Aaron Diamond Foundation.

MOLECULAR LESIONING OF OPIOID DELTA-NCX BINDING SITES IN RAT BRAIN BY ANTI-SENSE OLIGODEOXYNUCLEOTIDES. X.Y. Cha, H. Xu, K.C. Hopp0, E. Porco, J. LaB and P.B. Rothman. ICPS, IRP, NIDA, NIH Baltimore, MD 21224, 2LMC, NIDDK, NIH, Bethesda MD 20892. 3Department of Pharmacology, University of Arizona, Tucson AZ 85724.

Previous studies support the existence of two delta receptor subtypes called delta-cx and delta-ncx. Other studies have delineated the existence of additional subtypes termed delta-c1, delta-c2, delta-ccx, and delta-nx. These sites are assayed with [3H]DADL (10 mM TRIS-HEC, pH 7.4, 100 mM NaCl, 3 mM MgCl2, 2 mM GDP and 5 mM 2-mercaptoethanol) using rat brain membranes depleted of delta-ncx sites by pre-exposure with the delta-ncx-selective acetylating agent, (-)-trans-SUPERFIT or depleted of delta-cx sites by pretreatment with the delta-cx-selective acetylating agent, BIT. The recent cloning of a rat brain delta receptor raises the issue of the relationship between the multiple binding sites defined on the basis of our ligand binding studies and the molecularly defined delta receptor. To determine this, the antinonsense oligodeoxyribonucleotides complementary to the 5' end of the coding sequence of the cloned delta opioid receptor (nucleotides 7 to 26) and the corresponding sense oligodeoxyribonucleotides were administered i.c.v. twice a day for three days. Our data demonstrate that the antisense DNA selectively inhibited [3H]DADL binding to the delta-ncx site by 50%. Studies underway will determine effect of the antisense DNA on the two subtypes of the delta-ncx binding site.


The utilization of various agents to pharmacologically manipulate the opioid receptor system has been extensively studied using several radiolabeled techniques. In the present study, in vitro receptor autoradiography was used to characterize the anatomical distribution of [125I]-DAMGO binding sites in discrete brain nuclei of rats treated chronically with (+)RTI-14, a novel PCP site 2 ligand with moderate amine uptake inhibition activity, and of other potent amine uptake inhibitors. ALZET 2002 osmotic minipumps (0.5 ml/hr for 13 days) were filled with saline, (+)RTI-14 (1 mg/ml), cocaine hydrochloride (5 mg/ml) or imipramine (5 mg/ml). Regional brain sections (14 mm) at the striatal level were used to assess treatment effects on the mu opioid receptors. Initial findings demonstrate that (+) RTI-14 significantly decreased [125I]-DAMGO binding distribution in each specific nuclei measured. Chronic cocaine increased [125I]-DAMGO binding in caudate patches and matrix, while the imipramine-treated animals showed no changes from controls. Studies are in progress to confirm and extend these observations.


To characterize the establishment of opioid systems during development, we have begun to examine the distribution of 5 receptor mRNA at early postnatal stages of mouse development using in situ hybridization. In the developing nervous system, we have thus far detected restricted 5 receptor gene expression as early as postnatal day 1 (p1) in the postrema, pons-premotorum, and medulla oblongata, and have localized particularly intense hybridization to a band of cells near the brainstem, olfactory bulb, and peripheral nervous system. The distribution of 5 receptor mRNA levels as assayed by in situ hybridization has provided the developmental anatomical detail that complements prior receptor binding studies. In the hypothalamus at p4, for instance, 5 receptor mRNA is detected in specific cells of the luteinizing hormone area, in addition to the expression in the ventromedial hypothalamic nuclei similar to reported results from opioid binding studies. In addition, the pattern of high 5 receptor expression in subgroups of adult CA3/CA4 hippocampal neurons is already established at early postnatal ages. Although the levels of 5 receptor mRNA are low in all, cell groups, particularly in the ventromedial hypothalamic nuclei, relatively high transcript levels were detected in the pontine nuclei, reticular formation, and zona incerta nigra. The cellular resolution afforded by in situ hybridization has provided additional anatomical detail that complements prior receptor binding studies. In the hypothalamus at p4, for instance, 5 receptor mRNA is detected in specific cells of the lateral hypothalamic area, in addition to the expression in the ventromedial hypothalamic nuclei similar to reported results from opioid binding studies. In addition, the pattern of high 5 receptor expression in subgroups of adult CA3/CA4 hippocampal neurons is already established at early postnatal ages. Although the levels of 5 receptor mRNA are low in all cell groups, particularly in the ventromedial hypothalamic nuclei, relatively high transcript levels were detected in the pontine nuclei, reticular formation, and zona incerta nigra.


This study examined the magnitude of peripheral opioid antinociception in relation to 1) the development of inflammation, 2) µ-opioid receptor mRNA gene expression in dorsal root ganglia (DRG); 3) the number of peripheral µOR and 4) leakage of the perineurium of peripheral nerves. After Freund's adjuvant induced unilateral hindpaw inflammation in Wistar rats volume and temperature of the inoculated paws and, in parallel, anti-NOCICEPTIVE effects of DAMGO (µ-selective opioid agonist) increased significantly at 6 hrs, reached a maximum at 24 hrs and did not significantly change until 96 hrs. Doses of i.p. 8-KU, an irreversible µ-receptor antagonist, required to antagonize the anti-NOCICEPTIVE effects (IC50 values) increased linearly up to 96 hrs of inflammation. Using a expression protection assay, µOR mRNA levels were detectable in DRG 3-5 days after injury and displayed a trend towards an increase on the inflamed side by 24 hrs. Perineural leakage, assessed by horseradish peroxidase histochemistry, was apparent in late stages (60 hrs) of inflammation. In conclusion, gene expression, number and accessibility of µ-opioid receptors appear to be "up-regulated" during inflammation, resulting in a greater efficacy of peripheral acting opioids.
OPIOID RECEPTORS: LOCALIZATION


Mediation of opioid reinforcement has been associated with 5-opioid receptors in cortical-limbic structures. We utilized an antiserum shown to be specific for the 5-opiate receptor to determine whether topographical or ultrastructural localization could demonstrate a selective cellular substrate for these actions. Light microscopy examination among cortical areas revealed immunoreactivity (IR) in limbic-associated cortex to be distinguished by 1) lack of superficial laminar staining, 2) heavier deep laminar labeling and 3) sparse to moderate labeling of varicosity-laden fibers. Ultrastuctural analysis of insular cortex showed δ-opioid receptor labeling localized predominantly in small unmyelinated axons and terminals and in distal processes of axon terminals. In terminals, plasma membranes and synaptic vesicles were most intensely labeled. Additionally, dendritic labeling was detected and was most prominently associated with plasma membranes, smooth endoplasmic reticulum and occasionally perisynaptic densities. In a second portion of the study we examined the anatomical relationship between the δ-opiate receptor and endogenous opioids by combining immunoperoxidase and immunogold-silver methods, for localization of antisera directed against the δ-opioid receptor and [2°]-enkephalin (ENK) in single sections. ENK labeling was principally seen in large dense core vesicles (DCV) within axon terminals. Terminals appeared more homogenously distributed as compared to the δ-opioid receptor. In the regions that examined neither axon terminals nor dendrites were in contact with ENK neurone have shown detectable δ-IR. We conclude that in limbic cortex, δ-opioid receptors 1) are predominantly presynaptic modulators and 2) may be activated by opioid peptides released more distally from DCV. Supported by DA0660, DA01724, DA01657, DA01918 and Aaron Diamond Foundation.


Delta opioid receptor heterogeneity has been well established by pharmacological studies. Recently we have cloned a human δ opioid receptor by screening human cDNA libraries. It is suggested that this human δ opioid receptor matched the δ3 subtype, since it showed higher affinity to the δ3 selective ligand (NTB) than to that to the δ1 selective ligand (BNTX). Other cloned mouse and rat δ opioid receptors also shared δ3 receptor ligand binding properties. Thus, the δ3 subtype has not been cloned in any species.

In order to obtain the δ1 opioid receptor subtype molecular structure, one of the strategies is to find tissues containing δ1 rather than δ3 opioid receptor subtypes. Therefore, three interesting tissue membranes have been studied. First is the CXXBK mouse brain membrane, which was suggested to have a predominant population of supraoptimal δ3 opioid receptors, rather than δ3 opioid receptors by Raffa et al. (1992). The second is the guinea pig brain membrane, in which the δ1 selective antagonist BNTX showed 10-fold greater affinity (Ki = 0.1 nM) for [3H]PDPE binding sites (δ2) relative to those of [3H]DSG (δ3) (Portoghese et al., 1992). The third is the rat olfactory bulb membrane, in which the stimulation rather than inhibition of adenylate cyclase activity was observed (Ollano and Onali, 1992). Radioligand binding studies were performed in these three tissues, and the data will be presented. Supported in part by NIDA grants.


We have demonstrated previously that a population of small neurons in dorsal root ganglia as well as afferent fibers and terminals in the spinal cord are immunoreactive (IR) for delta (δ)-opioid receptors (Neuroreport 5:341, 1993). Presently, we report that most (μ)-opoid receptors share a similar distribution, and that both opioid receptors are restricted to a population of sensory neurons and axons not immunoreactive for neurofilament protein. Rabbit antisera produced against synthetic δ (IODS) or μ (MOR) opioid receptors were combined with a mouse monoclonal antibody (RT-97) directed against the 200 kD neurofilament protein subunit, which is a cytochemical marker for large sensory neurons with rapidly conducting axons. Dual color immunofluorescence was employed to test for the coexistence of opioid receptor- and RT-97 IR in sensory cells and axons. Within the DRG, MOR- and MOR-IR were confined almost exclusively to a population of small diameter cells and axons. In peripheral nerve, dorsal rootlets, and spinal gray matter, opioid receptor-IR was localized to small fibers. RT-97-IR was distributed to larger DRG cells and axons, and to peripheral nerve and spinal cord was found in large and small caliber fibers. Rarely did DRG- or MOR-IR coexist with RT-97-IR within the same neuronal elements, supporting the hypothesis that opioid receptors are localized to the terminals of nociceptive primary afferent neurons. Supported by NIH grant NS25658 and grants from NIDA.
THE RELATIVE DISTRIBUTIONS OF mRNAs ENCODING FOR MU AND DELTA OPIOD RECEPTORS AND FOR GLUTAMIC ACID DECARBOXYLASE (GAD) IN THE VENTRAL MESENCEPHALON. Darragh P. Devine*, Stanley J. Watson and Huda Aki. University of Michigan, Ann Arbor, MI, USA. 
Radioactive in situ hybridization was used to characterize the distributions of mRNAs that encode for mu and delta opioid receptors, and for glutamic acid decarboxylase (GAD) in rat ventral mesencephalon. The appropriate probe for each was used in combination with the delta and GAD mRNAs were labeled with 35S, and applied to serial sections of rat brain. GAD mRNA was most abundant in the interpeduncular nucleus (IPN). A moderate amount of GAD mRNA was found in the substantia nigra pars reticulata (SNpr) and substantia nigra pars compacta (SNpc) and Ventral Tegmental Area (VTA). Mu receptor mRNA was most abundant in the IPN, and low levels of mRNA were found in the SNpr, SNpc, and VTA. Delta opioid receptor mRNA was also most abundant in the IPN, with low levels throughout the SNpr, SNpc, and VTA. We are currently examining the possibility of cellular colocalization of these three mRNAs using double-labeled radioactive (35S) and non-radioactive (digoxigenin) in situ hybridization.

Activation of kappa (k) receptors diminishes excitatory transmission in the dorsal raphe and CA5 regions of the guinea pig hippocampal formation. Although many biochemical and physiological studies have suggested that k receptor-mediated neurotransmitter release is not yet evaluated, the anatomical localization of k receptors to pre- or post-synaptic processes has not yet been reported. We examined the distribution of a polyclonal rabbit antibody raised against a peptide from the C-terminal portion of a mouse k receptor. Specificity of the affinity-purified antibody was determined by Western blotting and immunolabeling of k receptors expressed in Xenopus oocytes. The antibody has been shown to be immunocytochemically in acronically fixed brain sections. Sections incubated in antibody preadsorbed with the antigenic peptide had no labeling by light or electron microscopy. By light microscopy, k receptor-like immunoreactivity (k-LI) was present in varicose processes in the dentate gyrus inner molecular layer, granule cell layer and hilus, and in CA3 and stratum lucidum and radiatum. By electron microscopy, k-LI was found in small unmyelinated axons and terminals, and was associated with large vesicular structures, cytoplasmic surfaces of small vesicles and plasma membranes. In the dentate gyrus, axons and terminals with k-LI contacted large dendrites and perikarya of presumed granule cells. In hila and in stratum radiatum of CA3, axons with k-LI were apposed to unbranched dendrites or other axons. In stratum lucidum, axons with k-LI were found occasionally in bundles of ribbon fibers. We were ultrastuctural data support physiological and biochemical evidence that k opioids modulate transmitter release from axons in guinea pig hippocampal slices. Supported by DA0269, DA0272, DA04123.

DELTA OPIOD RECEPTOR- AND ENKEPHALIN-IMMUNOREACTIVITY ARE SPATIALLY RELATED IN THE MOUSE CNS. L-H Lee*, TC Breit, RJ Dado, HH Loh, F-Y Law and R Eide. Departments of Cell Biology & Neuroanatomy and Pharmacology, University of Minnesota, Minneapolis, MN 55455
The cloning of a µ-opioid receptor (DOR) has permitted the raising of antibodies which recognize this protein. We previously reported that immunostaining for DOR in the dorsal horn of the spinal cord is strikingly restricted to axons and their terminals, and that these terminals are closely apposed to enkephalin-immunoreactive (ENK) boutons (Dado et al., NeuroReport 5:341-344, 1993). In the present study, we have examined the spatial relationship of DOR and ENK-nr fiber nerves in other regions of the central nervous system using histologic methods and montages produced from confocal microscopy. Nerve fibers and terminals with prominent DOR-ir were observed, for example, in the islands of Calleja and olfactory tubercle, the caudate-putamen, the globus pallidus and entopeduncular nucleus (n), amygdaloid nuclei, mammillary nuclei, the substantia nigra, interpeduncular n., raphe pontis, parabrachial n., of the solitary tract, lateral reticular n. and spinal trigeminal n. DOR- and ENK-nr fiber nerves have been described in these regions, however it is not clear if DOR-ir was found in the same elements as ENK-ir. Thus, DOR is unlikely to be an autoreceptor. In some regions, DOR-ir was abundant in areas which did not contain ENK-ir, and vice versa. These preliminary studies were in many instances immediately adjacent to each other, suggesting that the opioids may participate in interneuronal communication over significant distances. Supported by grants from NIDA.

A rabbit polyclonal antiserum to a peptide derived against a homogenate conjugate of the C-terminal amino acids of the µ opioid receptor. The antiserum produced high titters (1:4,000) when analyzed by ELISA, recognized purified µ opioid receptor protein in Western immunoblots, and stained in situ cells expressing the µ-opioid receptor. Microscopic examination of immunostained brain and spinal cord sections revealed that µ-opioid receptor immunoreactivity was present in fiber- and terminal patterns in amygdala, nucleus accumbens, globus pallidus, substantia nigra, medial habenula, substantia nigra, ventral tegmental area, periaqueductal gray, nucleus raphe magnus, nucleus tractus solitarius, zona spongiosa and substantia gelatinosa, while little was observed in neocortex or hippocampal formation. Neural cell bodies were immunostained in areas including hypothalamus. In situ hybridization studies of brain regions including thalamus revealed dups that were observed little tonomic immunoreactivity. No immunoreactivity was observed when preimmune or preadsorbed sera were used. These results directly show the distribution of the immunoreactive receptor in the rat central nervous system.

A MONOCLONAL ANTIBODY TO THE KAPPA OPIATE RECEPTOR. A.L. Brooks, K.M. Standifer, G.W. Pasternak, The Corzias Laboratory of Neuro-Oncology, Department of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021
Monoclonal antibodies (mab) were raised against Be(2)-C human neuroblastoma membranes which contain µ, δ, and κ opioid receptors. Over 5000 hybridoma cell lines were screened, with 2000 hybridomas testing positive against the Be(2)-C membranes. Screening against another neuroblastoma line lacking opioid receptors, left 98 positive for Be(2)-C mab. Several hybridomas showed high immunoreactivity to the Be(2)-C membranes, but only five of these clones, 8D6, 25G11, 13F3, 7G1, and 22H11 inhibited binding to the κ opioid receptor. Mab 8D6, inhibited up to 95% of 3H-NalBol (κ) binding with no effect on 3H-DAMGO (µ) or 3H-DPDPPE (δ) binding, illustrating the selectivity of mab 8D6 for the κ subtype. Mab 8D6 recognized κ receptors in mouse, rat, and calf brain homogenate binding assays. In addition, mab 8D6 blocked the inhibition of CAMP accumulation by NalBol but not morphine. On Western blots, mab 8D6 labeled proteins consistent with the putative κ receptor. Mab 8D6 also recognized a novel opioid receptor clone corresponding to the κ receptor. In immunocytochemistry studies the mab effectively labeled Be(2)-C cells but not cells lacking κ receptors. Mab 8D6 has also been effective in labeling brain slices, demonstrating its utility in establishing the distribution of κ receptors in the nervous system. Further characterization and purification of mab 8D6 is ongoing.

Antibodies to the δ-opioid receptor (DOR) and the µ-opioid receptor (MOR) were generated and used to map DOR- and MOR-immunoreactivity (i) in the central nervous system (CNS) of adult mice and rats (Lee et al., this meeting; Arvidsson et al., this meeting). In the present study, these antibodies were used to examine spatial relationships between these receptors and neuropeptides and other transmitters at the cellular level. Sera from rabbits immunized against amino acids 3-17 (Dado et al., NeuroReport 5:341-344, 1993) of the predicted sequence of DOR were used in conjunction with a monoclonal antibody to substance P (SP) for two color immunofluorescent staining and analysis. A high degree of colocalization between DOR and SP was seen in axons and terminals in many regions of the brain and spinal cord. Extensive colocalization was evident, for example, in the islands of Calleja and olfactory tubercle, the shell of n. accumbens, ventral pallidum, lateral septal nucleus, and ventral tegmental area. Results suggested that the apparent colocalization was not artifactual. Interestingly, high levels of DOR receptors occur on noradrenergic fibers originating from locus coeruleus. However, a high degree of colocalization was not observed for molecules which mark these systems. In summary, a summary was made of known regions which display different DOR- and SP-ir in axons and terminals, suggesting a presynaptic role for this receptor in regulating the release of SP and transmitters which coexist with it, in a wide range of systems. Supported by NIDA.

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OPIOID RECEPTORS: CELL BIOLOGY AND EFFECTOR MECHANISMS

T06.1
RECONSTITUTION OF A PURIFIED & OPIOID BINDING PROTEIN WITH PURE G-PROTEINS IN LIPOSOMES
Fan,L.*Q., Cisagliani,T.L.*, Dingus,J.X., Wilcox,M.A.*
Hildebrandt,J.M. and Simon,Y.F.*
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A functionally active & opioid binding protein (OBP), purified to homogeneity from bovine striatal membranes, has been reconstituted with purified G-proteins into liposomes containing the phospholipid, phosphatidylcholine. Liposomes were formed by precipitating the lipid-protein mixture with PEG and resuspending the liposomes in buffer. Most experiments were done with a highly purified bovine brain G-protein mixture, but individual purified Gs and Gq-proteins were also found to be effective. Functional reconstitution was evaluated by measuring stimulation of the GTPase activity of G-proteins by opioid agonists and high affinity agonist binding. Low-Km GTPase was stimulated by the & agonists DAMGO, DAGO, or levorphanol (max. 100% over basal), but not by DPDE or US50,488H. Stimulation was reversed by naloxone and b PAGE stimulation and naloxone reversal were stereospecific. The [3H]-DAGO binding observed in the liposomes with purified G-protein mixture and OBP had a Kd of 8nM, an affinity slightly lower than that seen in bovine striatal membranes, and was sensitive to inhibition by GTPyS and sodium. No high affinity delta or kappa binding was observed in the reconstructed system. The evidence from this study has shown that unexpected results suggesting that OBP is a & receptor.
(supported by grant DA-000117 from NIDA to EJS).

T06.2
INVOLVEMENT OF GUANYLATE 5'-TRIPHOSPHATE IN & OPIOID RECEPTOR-ACTIVATED POSTSYNAPTIC RESPONSES IN SUBSTANTIUM GELATINOSUM NEURONS. S.P. Schneider* and A.R. Light
Dept. of Physiology, University of N. Carolina, Chapel Hill, NC 27514-7545.
The involvement of the nucleotide 5'-GTP in the & receptor-activating & G-protein actions on substantia gelatinsa (SG) neurons was investigated using tight-seal whole-cell recordings from superfused transverse slices of hamster spinal cord. Bath application of the & receptor agonist [D-Ala2,N-Me-Phe4,Gly-ol5]-enkephalin (DAMGO) 1.5-7.5 mM caused a desensitization and decrease in neuronal input resistance (Ri) in 62% of SG neurons when the electrode internal solution contained 100 &M GTP and 2 mM ATP. However, at bath concentrations up to 10 &M DAMGO had little or no effect on the transmembrane potential or Ri of SG neurons using electrodes without GTP and ATP. DAMGO-activated responses were attenuated in SG neurons after dialysis with internal solution containing 2 mM ATP but no GTP. DAMGO activated a non-reversing membrane hyperpolarization in SG neurons recorded with electrodes containing 100 &M GTP-S, a nonhydrolyzable GTP analogue. SG neurons were less responsive to DAMGO following intracellular application of the analogue GDP-7-S (500 &M) which blocks &-protein activation. We conclude that intracellular GTP is necessary for &-opioid-receptor-mediated responses in SG neurons, consistent with the idea that anticonoperative effects of opioids are mediated through receptor-coupled second messenger systems involving G-proteins. The results resolve why DAMGO does not always evoke postsynaptic effects in superficial dorsal horn neurons in whole-cell recordings. Supported by NINDS grant NS23771 (S.F.S.), NIDA grant DA06440 (A.R.L.), and NIDODS grant DA4759013 (E.R. Peril).

T06.3
Dept. of Pharmacology, 1, Univ. of Washington, Seattle, WA and Div. of Biology* and CNS Program*, Caltech, Pasadena, CA.
Application of mu-opioid agonists evoked an increase in K+ conductance in Xenopus oocytes expressing the mu1 subtype receptor and the G-protein-gated, inwardly rectifying K+ channel (KGA). The amplitude of the response decreased during agonist exposure with a t1/2 of 8-22 min. In oocytes expressing both the mu1 and SHTA receptors, stimulation of either receptor resulted in heterogeneous desensitization of the subsequent response to the other. Injection of GTPyS (1 mM) increased the peak response to agonist but did not affect the rate of desensitization. Basal channel activity was significantly decreased in the absence of agonist and also desensitized at the same rate when the oocytes were clamped at -80 mV and bathed in high K+ (96 mM) solution. The above results indicate that the desensitization of the response occurred downstream of the receptor, possibly at the channel. Response desensitization occurred at the same rate if the cells were clamped either at 0 mV or with agonist in low K+ buffer (2 mM). The rate of desensitization was not significantly altered by any of the following treatments: removal of external Ca2+, prescaling the oocytes with 0.01 M CaCl2, increasing the extracellular Ca2+ (10 mM), staurosporine (50 &M), or cyclosporin A (50 &M). These results suggest that desensitization requires open channels but may not involve a calcium or phosphorylation-dependent mechanism or internalization of components of the transduction pathway. Supported by DA04123.

T06.4
KAPPA OPIOID RECEPTORS COUPLE TO INWARD RECTIFIER K+ CHANNELS WHEN COEXPOSED IN XENOPUS OOCYTES. D.J. Heady, M.P. Lim, H.A. Lester, N. Davidson and C. Chavkin.
Dept. Pharmacology, 1, Univ. of Washington, Seattle, WA and Div. of Biology* and CNS Program*, California Institute of Technology, Pasadena, CA.
Xenopus oocytes expressed kappa (k) opioid specific binding sites 5-14 days following injection of mRNA prepared from a clone of the mouse k opioid receptor (provided by David Grandy, Volum Inst., Portland, OR). Expression of k receptor (870 fmoles 1H-U59,869/mg membrane protein) was observed 7 days after injection of 5 ng mRNA/ococyte. The k receptor was previously shown to negatively couple to N-type Ca2+ channels. We find that the injection of k receptor mRNA with mRNA coding for an inward rectifier channel (KGA) resulted in oocytes that expressed k agonist-gated K+ currents. The K+ current (I69,593) activated a large (10-15 &A) inwardly rectifying K+ current with an EC50 of 200 &M. This agonist-gated K+ conductance was not observed in oocytes injected with mRNA for the k receptor or KGA channel alone. The k agonists, DAMGO (10-4 M) and U69,593 (1 &M) significantly potentiated the maximum response to U69,593. The acute response to U69,593 desensitized rapidly, with a t1/2 of 4 minutes following peak response. Supported by DA 04123 and DA 05610.

T06.5
ONTOGONY OF &-OPINATE INHIBITION OF ADENYLYL CYCLASE AND GTP MODULATION OF &-RECEPTOR BINDING IN RAT BRAIN. J.J. Li* and C. M. Kahn. Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27710.
Signal transduction pathways play a critical role in opiate receptor function, especially after chronic exposure. To understand the consequences of perinatal opiate exposure, it is necessary to characterize the coupling of opiate receptors to their respective signal transduction pathways during development. The present study investigated the inhibition of basal and forskolin-stimulated cAMP accumulation by DAMGO, a selective & agonist, in striatal membranes from 10 and 60 day old rats. The ability of Gpp(NH)p to shift striatal &-receptors from high to low affinity at these ages was also assessed.
DAMGO (0.1-10 &M) significantly inhibited basal and forskolin-stimulated cAMP accumulation by 25%, and 22%, respectively, in 60 day old rats. In contrast, DAMGO failed to inhibit significantly cAMP accumulation in 10 day old rats. Approximately 30% of striatal &-receptors were shifted to low affinity by Gpp(NH)p in 60 day old rats, whereas only 4% were shifted to low affinity in 10 day old rats. These data suggest that &-receptors in 10 day old rats are not coupled as tightly to effector systems as in 60 day old rats. These ontogenic differences in &-receptor coupling may prevent receptor function from adapting to chronic neonates especially vulnerable to the adverse consequences of chronic administration. (Supported by DA-02739 and MH-15177)

T06.6
CHP-212, A HUMAN NEUROBLASTOMA CELL LINE, EXPRESSES A HETEROGENEOUS POPULATION OF & OPIOID RECEPTORS
A.M. Babel,1 K.M. Standifer, L Cheng,1 L.J. Biedler1, and G.W. Pasternak2,3
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Identification of two & opioid receptor subtypes, termed &1 and &2, has been greatly facilitated by the use of specific agonists (DPDE and deltorphin II, respectively) and specific antagonists (BNTX and naltrindole, respectively). We have recently reported that the human neuroblastoma cell line, CHP-212, expresses five & opioid receptors, but does not demonstrate binding to &4 (DAMGO), &3 (H-U69,593) or &5 (H-NalPhe) specific ligands (Babel, et al., 1993). Competition binding assays in this cell line raise the possibility that the & receptor population in these cells is comprised of both &1 and &2 subtypes. The Kd values for the subtype-specific agonists and antagonists against H-Naltrindole were: DPDE 3.45 ± 0.56; deltorphin II 1.67 ± 0.33; BNTX 0.92 ± 0.24; naltrindole 0.21 ± 0.04; while the Hill coefficients were all less than one. In order to characterize the receptor proteins, 125I-&-endorphin was chemically cross-linked to CHP-212 membranes using BSOCS. SDS PAGE of the covalently labeled membranes reveals several specific bands. This cell line may be a useful tool in the study of the two & receptor subtypes.
OPIOID RECEPTORS: CELL BIOLOGY AND EFFECTOR MECHANISMS


Cloning of the delta opioid receptor offers a unique opportunity to characterize its functional coupling with effector systems in different cell lines. The rat growth hormone and prolactin secreting GH3 cells line expresses somatostatin (SRIF) receptors, G-protein-coupled families of K+ channels, but lack opioid receptors, assessed by ligand binding and cAMP assays. Therefore, GH3 cells would appear to be suitable for the expression of opioid receptors and for the investigation of their coupling to effector systems and hormone release.

A plasmid containing the entire coding region of the mouse delta opioid receptor (DOR) was transfected into GH3 cells. Stable expression of DOR was verified by immunocytochemistry and radioligand binding with [3H]D-Ala-Dleu-enkephalin (DADL). Twelve positive clones were analyzed for their ability to bind delta-specific ligands. One clone (GH-DOR-22 cells) was chosen for further analysis. GH-DOR-22 cells (D-PEN 2.5), enkephalin (DPDPE), etorphine and DADL (1-1000 nM) all dose-dependently displaced [3H]-dynorphine bound to GH-DOR-22 cells.

The ability of the transfected delta opioid receptors to inhibit adenylate cyclase was assayed by measuring cAMP levels in GH3-DOR-22 cells. Etorphine (1-1000 nM) dose-dependently inhibited adenylate cyclase in GH3-DOR-22 cells and the effect was blocked by naloxone. cAMP levels were also reduced by SRIF (0.01-5 μM) both in control and DOR-transfected GH3 cells.

The receptor's ability to modulate Ca2+ channels was investigated using the whole-cell voltage-clamp recording technique in both NG108-15 cells (from which DOR was cloned) and in GH-DOR-22 cells. While both DADL and SRIF inhibited voltage-activated Ca2+ channels in NG108-15 cells, neither was effective in the GH-DOR-22 cell line. In addition, both ligands failed to modulate K+-conducances in GH-DOR-22 cells. It is concluded that the delta opioid receptor can couple to adenylate cyclase in transfected GH3 cells, although this cell line may not be a suitable model for studying cloned opioid receptor coupling to ion channels.


Involvement of Gia in phospholipase C activation by metabotropic receptors in Xenopus oocyte expression system was studied. The current amplitudes were measured by injecting a molar equivalent of 15 δ opioid receptor (DOR) or 10 nM in the Xenopus oocytes injected with mouse δ-opioid receptor (DOR1) or M2 muscarinic receptor (M2) RNA were rapidly desensitized by repeated challenges of the agonist. Such a rapid desensitization was rescued by the addition of RNA of Gia and Gia2, but not Gia3, Gia4, Gia5, Gia6 or Gia7. In the oocytes injected with Gia2 and DOR1 or M2, the agonist-evoked currents were constant in amplitude upon at least 6 repeated challenges and these were completely abolished by the intracellular injection of 100 pmol of EGTA or 1 pmol of inositol 1,4,5-trisphosphate. When the chloride ion-concentration was reduced to one fourth, the I-V curve (or reversal potential) was shifted to the right (from -25 to 0 mV).

CO-LOCALIZED OPIOID AND GLUTAMATE RECEPTORS ON ASTROGLIAL CELLS - POSSIBLE REGULATORS OF SYNAPTIC TRANSMISSION. T. Thorstorp, P.S. Eriksson2, M. Nilsson, E. Hansen1 and L. Rönööck1,2. Institute of Neurobiology1 and Department of Neurology2, University of Göteborg and Department of Cell Biology, Faculty of Health Sciences, University of Linköping, Sweden.

Neurons and astroglial cells are the two major cell types in primary culture and respond differentially to opioid receptor stimulation using selective agonists. Cultured neurons were shown to respond with a substantial decrease in the cytoplasmic free calcium concentration in response to δ and μ opioid receptors. On the other hand, stimulation of δ and μ receptors, on astroglial cells in culture using DADL or U-50,488H resulted in a substantial increase in the cytoplasmic free calcium concentration. Cultured neurons were shown to be co-localized with opioid receptors on cultured astroglial cells. These effects were visualized through the use of the fluorescent calcium indicator fura-2. In astroglial cells in culture and acute slices of guinea pigs, astrocytes were identified by immunostaining with antibodies against glial fibrillary acidic protein (GFAP). The presence of δ-receptor mRNA was verified in cultured astroglial cells using RT-PCR and Northern blot. The present study suggest that cultured neurons from the cerebral cortex express μ, δ and κ receptors. Cultured astroglial cells from the cerebral cortex express co-localized δ, μ and κ receptors and acute dissociation of astrocytes and is under development to further investigate functional δ-receptors (i.e. calcium activating) to validate the relevance of the in vitro system. This might represent a novel mechanism contributing to the depressive action of opioids on synaptic transmission via decreasing the availability of calcium.
OPIDIO RECEPTORS: CELL BIOLOGY AND EFFECTOR MECHANISMS

T06.13

OPIOID RECEPTORS ARE DIFFERENTIALLY TARGETED IN THE NERVOUS SYSTEM AND IN A CELLULAR EXPRESSION SYSTEM. RI Dado*, AH Nakano, S Chakrabarti, LH Lee, M Riedl, U Arvidson, MW Westendorf, JH Loh, FY Law and E. Ribe, Departments of Cell Biology & Pharmacology, University of Minnesota, Minneapolis, MN 55455

The 8-opioid receptor (DOR) that has been cloned appears to be enriched in axons and terminals in spinal cord, where it may function as a presynaptic receptor (Dado et al., NeuroReport 5:341-344, 1994). Further examination suggests that DOR may be absent or even inversely related in axons. In contrast, the mu-opioid receptor (MOR) that has been cloned appears to be targeted to the plasma membrane of dendrites and cell bodies, and, in some cases, to axons (Arvidson et al., J. Neurochem. 62:1737-1743, 1993). Immunochemical studies were used to characterize this distinction more completely. COS-7 cells were electroporated with constructs which expressed DOR, MOR and the cloned 8-opioid receptor (DOR) behind a 8 amino acid epitope tag (NAG) followed by a 4 amino acid thrombin site. Cells were fixed 72 hrs after electroporation and stained with a monoclonal anti-TAG antibody. While both DORTAG- and KORTAG-transfected cells, TAG-immunoreactivity (4) was observed in the endoplasmic reticulum, the golgi and the plasma membrane. In contrast, in DORTAG-transfected cells, TAG-ir was restricted to the endoplasmic reticulum. The targeting of these receptors in axons was examined in sciatric nerves of rats which had been previously ligated under general anesthesia. DOR- and MOR-ir accumulated both proximal and distal to ligation sites at survival times ranging from 2-8 hrs. Taken together these results suggest that there are considerable differences among opioid receptors in their targeting. Supported by NIDA.

T06.14


Opioid binding assays were found in nuclear matrix preparations from NG108-15 neurohybrid cells. The opioid antagonist, -naltrindole, and -diprenorphine, display high affinity binding, while agonists bind with low affinity, if at all, to matrix nuclear preparations. Gpp(NH)p insensitivity of agonist binding and the absence of adenyl cyclase activity provided a molecular basis for the occurrence of uncoupled opioid sites in this fraction. Opioid inhibition of basal and forskolin-stimulated adenyl cyclase activity was found in nuclear membrane preparations. A decrease in agonist binding affinities and densities in P3 fractions was also observed under these conditions, whereas treatment of cells for forskolin potentiated both P2+ and nuclear matrix binding. Taken together these results support the hypothesis that nuclear membrane opioid receptors are newly synthesized molecules entrapped to the cell surface, whereas nuclear matrix contains internalized -sites.

T06.15


The purpose of the present study was to determine if the coupling pattern of a recently cloned -opioid receptor stably transfected in CHO cells to individual G protein subunits was different than that observed previously for B and -opioid receptors. Data presented in the current study indicate the successful expression of a -opioid receptor in CHO cells. This is supported by experiments which revealed that ligands with high selectivity for, but not for -opioid receptors demonstrated high affinity for the expressed receptor and were able to potentially and efficaciously produce inhibition of adenylyl cyclase activity. In addition, both -opioid agonists were able to induce dose-dependent increases in the incorporation of [35S]azidoanilido-GTP into four G protein subunits, three of which were identified as Gq, Gz, and Go. Further, the amount of -opioid agonists required to induce 50% maximal labeling of any individual G protein subunit was different. Although -opioid agonists produced equivalent maximal labeling of Gq, Gz, and Go, significantly less agonist-induced labeling was observed for an unknown G protein designated as Go. These results are similar to that observed previously for both and -opioid receptors and suggest that although all opioid receptors interact with multiple G proteins, this coupling is not selective for any individual G protein subunit.

T06.16

KAPPA OPIOD INHIBITION OF STIMULATED PHOSPHOHOSTIDES HYDROLYSIS IS NOT MEDIATED BY A DECREASE IN INTRACELLULAR CALCIUM. Dennis Paul*, Lema Minor and Naili Dunn, Department of Pharmacology and Center for Neuroscience and Alcohol and Drug Abuse, LSU Medical Center, New Orleans LA 70112.

Kappa opioid agonists close N-type Ca++ channels and attenuate stimulated phosphoinositide (PI) hydrolysis in neural tissue. PI hydrolysis is Ca++ dependent. Accordingly, we assessed the possibility that the kappa attenuation of stimulated PI hydrolysis is due to reduced intracellular Ca++. Rat hippocampal slices labeled with 86Rb were incubated with or without the Ca++ ionophore, BayK 8644 (10 μM) or A23187 (10 μM). These doses of ionophore completely uncoupled the ndependent PI hydrolysis. KCl (27.5 μM) or NE (30 μM) were added to stimulate PI hydrolysis. In addition, various concentrations of the kappa agonist U-50,488 were added. One hour later, the assays were stopped and PI hydrolysis was measured as the ratio of dpm released / dpm incorporated. U-50,488 dose dependently attenuated KCl-, NE- and carbachol stimulated PI hydrolysis. Assumptions of high intracellular Ca++ levels with BayK 8644 or A23187 did not affect kappa agonist attenuation of stimulated PI hydrolysis. Addition studies assessed kappa agonist effects on heart ventricle slices, a tissue in which kappa agonists increase intracellular Ca++. EKC and U-50,488 inhibited NE-stimulated PI hydrolysis dose-dependently. Together, these results provide evidence that kappa agonists do not attenuate PI hydrolysis by reducing intracellular Ca++.

T06.17

IDENTIFICATION OF TRANSCRIPTS ENCODING OPIOID RECEPTORS IN IMMUNE CELLS. Claire Gaydara-Raff, Frederic Simonin, Katia Befort and Brigitte Kieffer*, ESBS, Univ. Louis Pasteur, 67 Strasbourg, France.

Numerous in vivo and in vitro studies have shown that opioids modulate the immune response. A direct effect of opioids on immune cells has been posited. Pharmacological studies to demonstrate the existence of opioid receptors on these cells have been poorly convincing. We, and others, recently isolated cloned opioid receptor cDNA from immune cells. We designed opioid receptor subtype-specific primers, based on the known sequence of the cloned receptors, and used Reverse Transcriptase-Polymerase Chain Reaction followed by Southern hybridization, for the detection of opioid receptor transcripts. We have tested both mouse and human cells representative of the different components of the immune system. Our results indicate that kappa receptor mRNA is undetectable in several mouse T cell lines. We found low levels of expression of opioid receptors in thymocytes. Our data suggest that the cloned mu, delta and kappa receptor subtypes are not only present in nervous tissues, but also are expressed in immune cells.

T06.18

OPIOID-DEPENDENT TYROSINE PHOSPHORYLATION IN NEURONS. D. A. Mangora*, Department of Pediatrics, the University of Chicago Chicago, IL 60637.

In primary neuronal cultures derived from 6-day-old chick embryo cerebral hemispheres (ESC6) met-enkephalin elicits a transient activation of phospholipase D (PLD), which precedes a 2-fold increase in PKC activity. Opioid-dependent PLD activation involves the tyrosine kinase (TK) inhibitor herbimycin A, strongly indicating that the mechanism of opioid-dependent PLD activation involves tyrosine phosphorylation. Western blot analysis and immunoprecipitation of encoding neuronal opioid delta, mu and kappa receptors. The cloning of these receptors has provided molecular tools for the identification of the expression of opioid receptors in immune cells. We designed opioid receptor subtype-specific primers, based on the known sequence of the cloned receptors, and used Reverse Transcriptase-Polymerase Chain Reaction followed by Southern hybridization, for the detection of opioid receptor transcripts. We have tested both mouse and human cells representative of the different components of the immune system. Our results indicate that kappa receptor mRNA is undetectable in several mouse T cell lines. We found low levels of expression of opioid receptors in thymocytes. Our data suggest that the cloned mu, delta and kappa receptor subtypes are not only present in nervous tissues, but also are expressed in immune cells.
OPIOIDS: ANATOMY AND PHYSIOLOGY II

T07.1 PERIPHERAL EFFECTS OF NALOXONE IN MICE WITH GASTROINTESTINAL INFECTION. O. Pol and M.M. Puig. Anesthesiology Research Unit, IIMM, Universitat Autonoma de Barcelona, Barcelona, Spain. E-08003.

The aim of the present study is to evaluate the effects of opioid antagonists on gastrointestinal (GI) transit in mice with diarrhoea associated with intestinal inflammation. Diarrhoea was induced by p.o. administration of coxotin (CO), and demonstrated by weight loss (P < 0.01), and increased GI transit (< 0.05) when compared to animals treated with saline (SS). The presence of inflammation in CO was demonstrated by electron microscopy. GI transit was evaluated with a charcoal meal used as a marker. Diarrhoea was induced by coxotin (NO) or by methioideut, induced a significant increase (P < 0.05) in GI transit in CO but not in SS animals. The inactive enantiomer (+)NX had no effect either in CO or SS animals. The delta antagonist naltrindole (3 mg/kg) also induced an increase in transit (< 0.05) in CO animals, while M6 had no effect.

Our results suggest that CO diarrhoea induces a local release of endogenous opioids (EO) which play an inhibitory role in the physiological response to intestinal inflammation in mice. It is postulated that the EO's released, could be a mu or delta agonist.

T07.3 ACTIVATION OF BRAIN OPIOIDERGIC STRUCTURES IMPROVES CHRONIC EXERCISE STRESS IN MICE. J.R. Medvinsky, O. Borkin, V.K. Surpilly, O.B. Liyinsky. Central Sports Research Institute (Moscow, Russia). Research Center Mental Health (Moscow, Russia). Baylor/UT Southwestern Medical Center, Dallas, TX 75246.

Injection of opioids, direct electrical stimulation of the periaqueductal grey substance and transcranial electrical stimulation (TES)-"electroanalgia" increase velocity of conduction and improves gait in mice (Liyinsky et al. '85:86:89). Naloxone blocked the effects. Recently we demonstrated that TES improved the response to another type of stress: acute exercise stress (Medvinsky et al. '93). In this study, we investigated the effects of TES on exercise performance and locomotor activity of mice after chronic exercise training, consisting of 21 days of swimming 30 min/day. 72 mice (BALB/c) were divided into 4 groups (n=25). After 21 days of swimming and 3 days of TES (0.6mA, 30 min) prior to swimming, Group B (n=22) received TES and shum stimulation and Group C (n=22) received training only. The results demonstrated that TES had greater effects for mice receiving TES than for shum, as measured by swimming time vs. sham: 100:137.0 (p<0.01), vs. control - 80.3±4.1 (p<0.001). So, TES improved the adaptive response to chronic exercise stress. Further work revealed that the timing of TES, influenced the results. Mice (n=22) that received the last TES 20 hrs prior to swim, swam shorter than those receiving TES 6 hrs prior to swim but longer than sham (n=24). or control (n=23) mice (p<0.01). Difference in swim time between sham and control, which existed after 6 hrs (p<0.01), disappeared after 20 hrs. Naloxone (0.5 mg/kg ip. prior to TES) blocked the effects of TES. We concluded that brain opioidergic structures participate in the adaptation to chronic exercise training.

T07.4 SPINAL CORD AND BRAIN OPIATE RECEPTORS: DIFFERENTIAL EFFECTS ON LARGE-FIBER VERSUS SMALL-FIBER SOMATOSENSORY PATHWAYS. A.D. Legatt, C.F. Schroeder, L.B. Hollinger, and L.P. Selman. Departments of Neurology, Neurosurgery, and Orthopedic Surgery, University of Texas Southwestern Medical Center, Dallas, TX 75390.

During spinal instrumentation and subsequent sciatic nerve injury, morphine sulfate may be directed into the spine to the paracochlear space to provide postoperative analgesia, and bolus doses of intravenous narcotic may be given as part of the anesthesia regimen. Somatosensory-evoked potentials (SEP) to posterior tibial nerve stimulation are monitored to detect spinal cord compromise during this operation. We examined the effects of the narcotics on the cortical SEPs and on the SEPs recorded over the medulla (referred to as "cortical SEPs", and "cervical SEPs", are generated near the cervicomedullary junction. Bolus doses of intravenous narcotics did not alter the cortical SEPs, but did cause transient waveform changes and attenuation of the cortical SEPs. In contrast, intrathecal narcotics affected neither the cortical nor the cervical SEPs, though they did produce analgesia.

These SEPs predominately reflect activity in the large-fiber (dorsal column/lemniscal) somatosensory pathways. The differential effects of the narcotics on the cortical SEPs demonstrate that the large-fiber afferent somatosensory pathways are influenced by opiate receptors in the brain but not by those in the spinal cord. Since intrathecal narcotics produce analgesia, spinal cord opiate receptors affect the small-fiber (pain), but not the large-fiber, somatosensory pathways within the spinal cord.


Dynorphin (DYN) facilitates conditioned place aversion and reduces locomotor activity through mechanisms possibly involving direct activation of target neurons or presynaptic release of catecholamines or Substance P (SP) in the nucleus accumbens (Acb). We examined the ultrastructural substrate underlying these actions by combining, in single sections of rat tissue, immunoperoxidase and gold-silver labeling for dynorphin (D) and either the dopamine- or the Substance P-immunoreactive neurons. DYN-labeled perikarya were spherical in shape, contained unindented nuclei and were closely and dently, the other type of structures were small, of which some also contained SP labeled perikarya. Smooth endoplasmic reticulum and coated vesicles could also be identified in the cytoplasm and the synaptic vesicles. A major feature of these neurons was the presence of synapses on the axon terminals of other neurons. Certain Type DYN terminals were located in the regions of the Acb where SP was also found. The ultrastructural features of DYN terminals include the presence of small and large core vesicles and their prominent formation of synaptic structures. The DYN terminals formed primary synaptic contacts with small and large core vesicles and their prominent formation of synaptic structures. The DYN terminals formed primary synaptic contacts with small and large core vesicles and their prominent formation of synaptic structures. These terminals also formed secondary synaptic contacts with other DYN terminals (1:40:370) and other terminals that were unlabeled (14%), TH-labeled (90%), DYN-labeled (14%) or SP-labeled (11%). We conclude that DYN may be involved in the transmission of different types of axons to the Acb. In conclusion, DYN may be involved in the transmission of different types of axons to the Acb.

T07.6 REGULATION OF DYNPHORIN (DYN), TYROSINE HYDROXYLASE (TH) AND [Met]-ENKEPHALIN (ME) IN NEURONAL/GliaL CO-CULTURES FROM RAT HYPOPHALAMUS G-CW; M.K. McMillian and J.S. Hong LMIN/NIEHS/NH, RTP, NC 27709.

Hypothalamic neurons contain the highest concentration of dynorphin in the brain and this peptide may be an important modulator of feeding behavior as well as contribute to regulation of pulmonary hormone secretion. Using a mixed hypothalamic cell culture model (neurons, glia, neurons from E16 fetuses), we have examined the second messenger pathways involved in regulation of DYN, and have compared DYN responses to changes in TH-immunopositive neurons (>10^7) cell number and ME release. Elevating cAMP with forskolin (FSK,10^7 M) increased DYN levels and also increased TH cell number. Dexamethasone (DEX,10^-7 M) increased dynorphin production, but did not affect ME release or TH cell number. FSK + DEX produced a near additive effect on DYN, but had a more pronounced effect on ME release (previously shown to be glia derived). Depolarization with KCl (45 mM) more effectively increased TH cell number than DYN, and did not affect activation of PKC with phorbil ester (10^-7 M). DYN is regulated differently than TH and ME in these hypothalamic cultures.

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T07.7

DEVELOPMENT OF OPIOID TOLERANCE IN THE tS TIs ASSOCIATED WITH GENERAL CHANGES IN NEURONAL RESPONSIVENESS. C.J. Malanga*, W.W. Fleming and D.A. Taylor. Dept. of Pharmacology, West Virginia University School of Medicine, Morgantown, WV 26505-9223.

Chronic morphine exposure results in non-specific changes in the sensitivity of guinea-pig nucleus tractus solitarius (nTS) neurons to inhibitory agonists. While a 56-fold rightward shift of the dose-response curve to morphine was observed, no significant shift of the dose-response curve to morphine was seen with cumulative drug applications. One explanation for the lack of altered sensitivity to morphine is acute desensitization, which can be avoided by application of single drug concentrations to spontaneously-active nTS neurons. Using standard extracellular electrophysiological techniques in brainstem slice preparations, we have found that the application of forskolin (1 μM) resulted in an 80±7% inhibition of firing in control and a 47±5% inhibition of firing in chronically morphine-exposed neurons, indicating a significant (P<0.05) 41% reduction in response to morphine in the morphine-treated group. Similarly, application of muscimol (0.3 μM) to the same nTS neurons inhibited firing 57±11% in controls and 30±8% in morphine-treated preparations, indicating a significant (P<0.05) 47% reduction in response to muscimol in morphine-treated animals. Furthermore, elevation of extracellular K+ (normal ACSF=5.0 mM [K+]o; depolarizing ACSF=7.2 mM [K+]o) resulted in an excitation of 85±13% in control neurons and an excitation of 146±47% in neurons from the morphine-treated group. Together, these findings suggest that 1) the in vitro nTS/brainstem slice preparation is an appropriate model system in which to study the development of opioid tolerance, and 2) the opioid tolerance in the nTS is associated with a non-specific, general change in the physiology of these neurons. Supported by NIDA grant DA 03773.

T07.9

PROPOIOMELANOCORTIN EXPRESSION IS REDUCED BY AN ANTISENSE OLIGONUCLEOTIDE. S. Spampinato, G. Campagna, L. Carboni and S. Ferrri. Dept. of Pharmacology, Univ. of Bologna, 40126 Bologna, Italy.

Gene expression in mammalian cells can be suppressed by oligonucleotides complementary to the target mRNA. This strategy was explored as a means of preventing translation of the proopiomelanocortin precursor (POMC). The synthesis of the POMC-derived peptides adrenocorticotropic hormone (ACTH) and β-endorphin (β-END) was markedly reduced by an oligodeoxynucleotide (ODN) complementary to a region of β-END mRNA in AT-20 cells, which retain many of the differentiated phenotypes of corticocytes; this treatment did not affect the steady-state levels of POMC mRNA. Antisense ODN was stable in cell culture medium for 24 h and cellular uptake was low (<2.5% of the added ODN), however, the intracellular levels of the ODN were sufficient to form a rhonucleoace-resistant duplex with complementary cellular mRNA. Addition of ODN to the cell culture did not affect cell viability and proliferation. Microinjection of the antisense ODN in the rat hypothalamic arcuate nucleus (0.625 μg/50 μl), where the majority of POMC-expressing brain penkaryas are located, significantly reduced ACTH-immunopositive neurons in the medio-basal hypothalamus ([140±87 vs 250±940) in vehicle-treated rats, p<0.01; n=7] and antisense ODN-treated rats showed substantially less of the grooming behavior usually observed in a novel environment. Changes of nocioceptive threshold, body temperature and knowledge of plasma levels of anterior pituitary hormones in rats exposed to antisense oligonucleotide will be presented.

T07.10


Oxytocin neurons in the suprachiasmatic nucleus (SCN) develop dependence on morphine given intracerebroventricularly (i.c.v.) over five days (up to 5 μg/kg) as revealed by naloxone (NLOX) precipitated morphine withdrawal which increases oxytocin firing rate by 3.5 fold. We have studied the importance of the noradrenergic input for the expression of this withdrawal excitation (1) NLOX (5mg/kg) precipitated withdrawal-induced Foex expression in tyramine hydroxylase expressing neurons in the nucleus tractus solitarius. These neurons are known to project to the SCN and mediate the response of oxytocin neurons to sympathetic cholinergic nerve terminals (CCK). (2) Electrophysiological extracellular recording was carried out in urethane anaesthetized rats. Oxytocin neurons were identified by firing pattern, antidromic activation and response to CCK. The extrastimulus to i.v. CCK injection was similar before (mean±S.D.: 1.060±0.39 Hz increase, over 5 mins) and after NLOX in morphine-inhibited rats (1.18±0.46 Hz, n=11), though smaller than in morphine naive animals (3) Clonidine (2.5 mg/kg, i.v.), an α2 adrenoceptor agonist, attenuated the rise in oxytocin secretion after withdrawal (mean±S.E.M.: vehicle: 702±186 pg/ml vs. + clonidine: 303±113 pg/ml after 10mins, n=7, p<0.05, one-way t-test) (4) Electrical activity in oxytocin neurons after withdrawal was reduced by both acute (25μg) and continuous (0.32 to 1.62 μg/ml for >20 minutes) administration of i.v. α2 adrenoceptor antagonists such as benoxatn. This effect could be reversed by simultaneous infusion of the α2 adrenoceptor against phentolamine. These results indicate that an excitatory, or permissive, noradrenergic input to oxytocin neurons drives the expression of opiate withdrawal-induced excitation.

T07.11


We utilized whole cell and intracellular recordings from slices of rat brainstem to study the effects of opioids on LC neurones. The amplitude of the [net]- enkephalin-induced outward current (I_m) was larger and E_m was more negative in slices cut in the horizontal plane than in the coronal plane. Morphological analysis with neurobiotin indicated that cells from horizontal slices had a more extensive dendritic arborization than from the coronal plane. When the pipettes were filled with Cs gluconate, I_m was still outward, did not reverse polarity even at strongly negative potentials and was blocked by BaCl_2. When recordings were made from 2 neurones, BaCl_2 and TTX induced synchronous oscillations (mean peak potential. LC basic properties were not affected by carbenoxolone, a gap junction blocker which reduced extracellular potential to the right and prevented the Ba-induced oscillations. The results suggest that I_m is mediated by an increase in K_m only. Dose predictions for K result from poor space clamp which may be due to electrotonic coupling among LC cells.

T07.12

PAIN-PRODUCING SUBSTANCES AUGMENT IA IN NODOSE GANGLION NEURONS. S. L. Ingram1 and J. T. Williams2. Department of Pharmacology1 and Vollum Institute2, Oregon Health Sciences University, Portland OR 97201.

Our previous work in the nodose ganglion has shown opioid inhibition of forskolin-stimulated IA in a small subpopulation of nodose neurones. Pain-producing substances which are known to increase cAMP levels (PGF_2α, bradykinin, and 5-HT) also elicit an inward current (at -60 mV) in a subpopulation of neurones. The mean amplitude of the inward currents are as follows: PGF_2α (1μM), 120±39 pA (n=4); bradykinin (1μM), 263±48 pA (n=7); and 5-HT (10μM), 122±12 pA (n=5). Currents were blocked in the currents were blocked in 3/7 neurones regardless of drug applied. Thus, we have preliminary evidence that agents other than forskolin are effective in augmenting IA. In an attempt to further identify responsive neurones on the basis of conduction velocities, intracellular recordings from guinea pig isolated nodose ganglion preparations were made. In 75% of neurones with conduction velocities >1.5 m/s (n = 28), only 35% expressed an IA current. In contrast, 83% of the neurones with conduction velocities >1.5 m/s had an IA current. These results suggest that IA neurones may be regulated by both pain-producing substances and opioids through opposite actions on adenylyl cyclase. (Supported by NIDA Grants DA00143, DA01863, and DA07262.)
707.13

Chronic treatment of rats with opioids and MK-801 (MK) inhibits the development of tolerance and physical dependence in intact animals. The mechanism of this phenomenon is obscure. Use of the isolated spinal cord of rats eliminated neonatal potential interference from behavioral effects. Newborn rats were treated with either morphine (M) or MK + M in incrementally increasing doses for 3 days. The superfusion bath. Nociceptive reflexes elicited by electrical stimulation or capsaicin were inhibited by M (10 nM - 10 μM) in controls (18 ± 10.5% S.E.M. depression of capsaicin response at 10 nM to 81 ± 3.4% at 10 μM) in cords of neonates treated with chronic M. Additional doses of M did not depress nociceptive reflexes indicating the development of tolerance. The development of dependence evidenced by increases in naltrexone-induced spontaneous activity was not affected by co-treatment with MK. Thus, MK combined with chronic treatment with M failed to block the development of tolerance and physical dependence in this model.

We conclude that the spinal cord has to be mature and/or act in synergy at least with the periphery (spinal) for MK inhibition of development of tolerance and physical dependence to be realized.

707.15
EFFECTS OF DAMGO, HALAIXONE AND ACETYLCHOLINE ON THE FIRING OF MEDIAL PREFRONTAL CORTEX NEURONS. J.L. Giacchino and E. Henssler. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Extracellular recordings were made from fifty-six spontaneously firing neurons of the medial prefrontal cortex (mPFC) in halothane-anesthetized rats. Areas investigated include the dorsal anterior cingulate, prelimbic, and infralimbic components of the prefrontal cortex. Intra-cranial applications of DAMGO demonstrated that systemic morphine decreases neuronal activity in the mPFC, and that this effect can be reversed by systemic naloxone. As the mPFC is suggested to function in reinforcement pathways and drug-related behaviors in the rat, the action of opiates, specific opioid receptor agonists and antagonists at the cellular level is of interest. Immunocytochemical localization of the selective mu agonist DAMGO (1 μM) decreased firing of most mPFC neurons studied. This drug effect was typically slow in onset and often prolonged (approx. 3-10 min). This effect could be blocked or reversed by naloxone (2 μM). In most cases, the activation of mPFC neurons was not apparent until new firing patterns emerged which had no effect alone on most mPFC neurons. As expected, acetylecholine (100 mU) increased firing in the majority of spontaneously active mPFC cells. In addition, ACh pulses elicited transient increases in cell firing during DAMGO-induced depression, and also during concurrent application with naloxone. Even when mPFC neuronal firing was completely suppressed following DAMGO, ACh was able to elicit firing, implying a receptor specific effect. These results suggest that activation of mPFC neurons may hyperpolarize mPFC neurons and/or may function presynaptically to inhibit excitation (Supported by SOAC #3-5-94217 and HIDA #3-5-94213).

707.14

SNC-80 is a new, highly selective non-peptide 5-opioid (Calderon et al., J. Med. Chem. in Press, 1994). Unlike other peptide and non-peptide opioids, high I.C.V. doses of SNC-80 (up to 800 μg) fail to elicit EEG seizures or convulsant activity in rats. In contrast, SNC-80 causes a complex behavioral response consisting of initial, brief periods of stupor followed by prolonged episodes of intense hyperactivity associated with marked EEG theta-driving (unpublished observations). We have begun to assess the effects of SNC-80 on a neuronal culture model of glutamate excitotoxicity. In the present study SNC-80 was added to primary rat cortical cultures immediately prior to 80 μM glutamate. Neurotoxicity and/or neuroprotection was assessed 18 h later. LHN measurements and morphological evaluation revealed that SNC-80 produced a concentration dependent neuroprotection. Compared to glutamate alone, 5, 20, and 40 μM SNC-80 protected neurons to the extent of 16, 30, and 60%, respectively. Intracellular calcium ([Ca]i) was monitored as an indicator of glutamate-induced neuronal injury in these same cultures. While glutamate consistently produced a sustained increase in [Ca]i, in 83% of the neurons analyzed, treatment with SNC-80 (50-100 μM) resulted in a shift in the [Ca]i, dynamic such that greater than 60% of the neurons demonstrated only briefer or transient increases in [Ca]i, an event previously shown to correlate with less toxic concentrations of glutamate (Busatto et al., NeuroReport, 1992). These results demonstrate that SNC-80 is neuroprotective in vitro and suggest a potential utility of selective 5-opioids for attenuating CNS injury.

707.16

The distribution of dynorphin mRNA in brain and spinal cord of adult male rats was studied in situ hybridization histochemistry. Intact or colchicine-treated animal brains were perfused with paraformaldehyde (PF) and sectioned with a vibratome or (f) frozen on dry ice, cut on a cryostat, thaw-mounted, and post-fixed with PF. Free-floating vibratome sections and mounted slides were treated with triphenyltetrazolium, then dehydrated with ethanol. While vibratome sections were rehydrated, cryosections were air-dried after dehydration. Sections were hybridized with a 35S-UTP-labeled probe (bp 100-835 of dynorphin cDNA), incubated overnight at 55 C and stringently washed to remove nonspecific label. Subsequently, vibratome sections were mounted on gelatin-coated slides. Slides were dipped in liquid photographic emulsion and exposed for ten to twenty days. Evaluation of autoradiograms revealed that the location of dynorphin mRNA was in good agreement with the location of the peptide detected by immunocyto-chemistry. However, we were able to detect mRNA in several regions of the central nervous system in which no peptide was reported. These include the olfactory bulb, the island of Calleja, posterior thalamic nuclei, CA1 and CA2 regions of the hippocampus, optic tectum, spinal trigeminal nucleus, etc. In addition, the number of mRNA-containing perikarya was higher than the number of peptide-containing perikarya previously observed in cerebral cortex. The use of 30 μM vibratome sections resulted in a shorter exposure time and less background than the use of 16 μm cryosections. Colchicine-treatment had no significant effect on the level of dynorphin mRNA.

707.17
HILAR MEDIATED EXCITATION AND LTP OF THE DENTATE GRANULE CELL. U. Werman*, M. Simmons*, C. Charyk. Departments of Anesthesiology and Pharmacology, University of Washington, Seattle, WA 98195.

Kappa agonists inhibit excitatory neurotransmission and long-term potentiation (LTP) in the hippocampus via a decrease in amino acid release from both hippocampal perforant path and mossy fiber terminals. We now report a third site of kappa opioid inhibition of excitatory neurotransmission in the hilar region of the dentate gyrus.

Extracellular recordings were made in the granule cell layer of the dentate gyrus of the guinea pig hippocampal slice. The hilar slice was selectively stimulated by a probe cut through the molecular layer to sever the perforant path and placing the stimulating electrode in the molecular layer in order to record the stimulating electrode. In the presence of bicuculline (10μM), stimulation (0.3 μs, 50-300 μA) of the hilar pathway elicited granule cell population spikes whose amplitudes were decreased by the kappa agonist, U69593 (10μM) in a non-competitive manner. LTP in this pathway was blocked by the NMDA antagonist, APV (25μM), and by U69593. Without bicuculline, no population spikes were elicited from the hilar path and no LTP could be induced. However, following LTP, population spikes were evident with or without bicuculline.

Thus kappa opioids modulate neural excitability in at least three distinct regions of the hippocampus. Although the hilar pathway studied here is normally under strong GABAergic control, plastic changes appear capable of overriding GABA inhibition - perhaps leading to hyperexcitation. [Supported by NIEHS and the Foundation for Anesthesia Education and Research with a grant from Abbott Labs.]

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
OPIOIDS: THE POTENTIATION OF THE NMDA RESPONSE INDUCED BY SIGMA LIGANDS IN THE RAT DORSAL HIPPOCAMPS I. N. Lavoie, R. Gogoi, and G. Debonnel. Neurobiological Psychiatry Unit, McGill University, Montreal, Quebec, Canada, H3A 1A1

Sigma ligands modulate the response of dentate neuronal nuclei to NMDA in the CA3 region of the dorsal hippocampus. It has also been found that CCK is involved in the effects induced by sigma ligands on cysolic motility. The present experiments were undertaken to determine the interaction in the CNS. Using five-barrelled glass micropipettes for extracellular recordings and microphotonics, we evaluated the effects of different CCK and CCK receptors antagonists on the modulation of sigma ligands activity, induced by the intravenous administration of low doses of the sigma ligands DTG (+pentazocine and JO-1784 on rat CA3, dorsal hippocampus) and pyramidal neurons. The potentiation of the NMDA response induced by these sigma ligands was abolished by the specific CCK receptor antagonist SR 27897, but not by the CCK receptor antagonist CI-988. CCK-85, applied as a low antagonist, insufficient to inhibit an increase of the firing activity by itself, markedly potentiated the response of NMDA, without affecting that of QUIS. SRI 27897 applied during the potentiation of the NMDA response by CCK-85, markedly reduced this potentiation. In contrast, the selective CCK receptor antagonist CI-988 applied microiontophoretically, and PD 153515 administered intravenously (3mg/kg), failed to abolish the effect of CCK-85 on the NMDA response. Our results suggest that the potentiation of the NMDA response of hippocampal pyramidal neuron by sigma ligands involves the activation of CCK receptors. Since CCK-85, by itself, potentiates markedly and selectively the NMDA response, these results also suggest that, in the CA3 region, sigma ligands might induce their modulatory effect on the NMDA response by promoting the release of CCK-85.

ENDOGENOUS DYNORPHIN RELEASE IN THE GUINEA PIG DENTATE MOLECULAR LAYER IS MEDIATED BY L-TYPE CALCIUM CHANNELS. M. L. Simmons1, O. W. Terman2, S. M. Gibb3, and C. Chavkin4. Dept. of Pharmacology and Anesthesiology, University of Washington, Seattle, WA 98195.

In the dentate molecular layer, dynorphins are released from granule cell dendrites to inhibit glutamate release. In addition, receptors on perforant path afferents. In the CA3 region, dynorphins are released from, and act upon, mossy fiber terminals. In both regions, dynorphins also have been shown to potentiate the release of LTP. The present study investigated the roles of CA3 channel subtypes in mediating the release of endogenous dynorphins. High frequency stimulation was applied to granule cells to induce dynorphin release, which was detected as a 20-30% depression of the field EPSP that lasted 2-15 minutes. In the dentate molecular layer, the L-type Ca2+ channel blockers nifedipine (10 μM) and PN20-110 (5 μM) inhibited the dynorphin-mediated depression. These drugs did not alter the effect of kappa receptor activation by exogenous agonist (1 μM U69593) application, nor did they affect the field EPSP responses themselves. Therefore, the L-type channel blockers inhibited dynorphin release without inhibiting the action of dynorphin or the release of glutamate. Furthermore, nifedipine and PN20-110 facilitated the induction of LTP in the dentate gyrus. This result is consistent with our previous studies showing that the kappa opioid receptor antagonist, norbinaltorphimine, also facilitates the induction of LTP in contrast to the effects observed in the dentate gyrus, the L-type channel blockers had no effect on dynorphin release from mossy fibers in CA3. Supported by DA04123.


We have previously reported that central, but not peripheral, administration of beta-endorphin (BE) to infant rats markedly decreases basal levels of ODC (α and e-phenylthiocarbamyl) activity throughout the body, as well as tissue ODC responsiveness to classical trophic factors. Based on these and other observations we have hypothesized that endogenous BE plays a prime role in controlling postnatal development. The question remains as to whether perinatal exposure to opioid agonists causes a pattern of developmental effects similar to those produced by BE.

In the current study we found that, like BE, intracerebroventricular (i.c.v.) administration of 2 μg of morphine to 1â†“-old rats decreases basal levels of liver ODC activity. In addition, hepatic ODC responsiveness to subcutaneously (s.c.) administered insulin (I) or growth hormone, two important trophic hormones, was almost totally arrested in pups pretreated i.c.v. with morphine. Similarly, morphine inhibited the half-life of exogenously administered I. As expected, hypoglycemia in rats injected with morphine i.c.v. plus I was observed in pups given I alone. Equivalent results were obtained in animals given morphine s.c.

The data strongly suggested that the developmental disabilities commonly observed in infants born to opiate-addicted mothers could result, at least in part, from an exacerbation of the effects of endogenous opioids on tissue ODC expression and on insulin/glucose metabolism. (Supported by USPHS Grant NS25738).

ENDOGENOUS OPIOID PEPTIDES MEDIATE THE SNUCKING INDUCED PROLACTIN INCREASE IN POST-PARTUM FEMALE RATS. S. N. Klosterman, D. Puntney, P. Callahan, and J. Janet, Miami University, Dept. of Zoology, Oxford, Ohio 45056.

We have reported that beta-endorphin is a potent stimulus for prolactin secretion in virgin female rats (Klosterman et al., Neuroendocrinology 57:275, 1993). Doses as low as 25 ng were capable of producing a prolactin secretory response that mimicked the prolactin response to suckling in post-partum female to beta-endorphin treated rats. In addition, intracerebroventricular (i.c.v.) administration of the opioid peptide (EOP), into the nucleus accumbens or dynorphin, both of which are regions involved in the regulation of prolactin secretion during lactation. Lactating, female, Sprague-Dawley rats between 3 and 6 days post-partum were used in all experiments. Animals were surgically implanted with chronic intraventricular (i.vt) cannulae into the lateral ventricle on day 2 post-partum. At least a 5 day recovery period and one day prior to the experiment, animals were implanted with chronic jugular cannula to facilitate blood withdrawal. On the day of the experiment, dams were separated from their pups for six hours prior to i.v administration of specific antisera to met-enkephalin or dynorphin. Blood samples were withdrawn immediately prior to antiserum injection and at 15 minute intervals, up to 1 hour, after the onset of suckling. Antiserum to each one of the EOPs effectively blocked the suckling-induced Prolactin increase. These results indicate that met-enkephalin and dynorphin are both important in the suckling-induced Prolactin increase. The mechanism(s) responsible for this action is not known. It is also not clear which receptor subtype mediates this response or if there is interaction among the different EOP and/or their receptor subtypes. (This work was supported by NIH grant # R15 HD30375-01 to JI and PC).

MORPHINE TOLERANCE/DEPENDENCE: REVERSAL FROM INHIBITION TO ENHANCEMENT OF cAMP FORMATION. L. Wang1 and A.R. Gunster, Dept. of Biochemistry, SUNY at Stony Brook, NY 11794, U.S.A.

This laboratory has previously demonstrated that sufentanil can produce a naloxone-reversible increase or decrease in the stimulated formation of cAMP in the myentric plexus, depending on the concentration of opioid employed. Low doses of opioid (1010 M) enhance whereas higher concentrations (107 M) inhibit the magnitude of cAMP formation. On the basis of these results, we also hypothesized that increases are positively as well as negatively coupled to adenyly cyclase. In the present study, the effect of chronic in vivo morphine exposure on the balance between opioid excitatory and inhibitory actions, and opioid-activated phosphodiesterase and cAMP formation. In fact, the final assay is not significantly different from that which occurs in opiate naive preparations in the absence of exogenous opioid. This indicates the development of tolerance to the negative modulation of stimulated adenylate cyclase by sufentanil. However, in 'addicted' tissue, the magnitude of increase in cAMP formation produced by electrical stimulation, in the presence of an inhibitory concentration of sufentanil, is significantly larger than that observed in 'addicted' tissue that had been washed in morphine-free Krebs' or Krebs' solution containing naloxone. Thus, the balance between the magnitude of stimulation-induced cAMP elevation in opiate naive vs 'addicted' tissue in the presence of sufentanil, is due to the ability of an originally inhibitory concentration of opioid to enhance or facilitate stimulated formation of cAMP. This evidence suggests that the opioid inhibition of stimulated cAMP formation results not only from the loss of inhibitory potency but from the reversal to enhancement.
THE EFFECT OF HALOPERIDOL ON PREPROENKEPHALIN GENE EXPRESSION IN THE RAT ANTERIOR PITUITARY.

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Department of Physiology, University of Hong Kong, Hong Kong.

To study the effect of haloperidol on the gene expression of preproenkephalin, rat anterior pituitary tissue, at Day 7 postpartum, was injected with 2mg/Kg haloperidol i.p. The anterior pituitary was homogenized in AT buffer using RNAse-free DNase and RNAse. Total RNA was obtained after digestion with proteinase K and the equivalent of about one-sixth of an anterior pituitary was assayed for preproenkephalin mRNA using solution hybridization. Nonspecific hybridization. Standards and P-32 labeled riboprobes were prepared from linearized plasmid DNA's (courtesy of Dr. J. Hong), using SP6 polymerase. An action probe (gift of Dr. D. Autelitano) was used to normalize the value, which was expressed as a percentage of preproenkephalin mRNA per pg actin mRNA. It was found that haloperidol treatment resulted in a significant decrease of preproenkephalin mRNA contents in the anterior pituitary, and this correlated well with the decrease of net-enkephalin levels as measured by RIA. Dopamine receptor blockade may therefore lower the net-enkephalin level in the anterior pituitary through a decrease in preproenkephalin synthesis.

TREATMENT WITH ANTISENSE OLGODEOXYNUCLEOTIDE TO THE CLONED PATTERNS OF ENKEPHALIN IN VIVO INDUCES ANTI-OPIOID POTENCY.

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CCK may function as an endogenous anti-opioid. Thus, intracerebroventricular (i.c.v.) L-505,250 (CCK receptor antagonist) increases the antinociceptive potency of i.c. morphine. In mice, the modulatory effect of CCK antagonist is not the direct morphine antinociception, but as shown to increase the antinociceptive response of naloxone. In the present study, the i.c.v. administration of CCK antagonist (100ng/mouse) resulted in a significant decrease in the antinociceptive response of morphine, as assessed by the tail-flick assay.

DIFFERENTIALLY EXPRESSED RELATIONSHIPS BETWEEN ENKEPHALIN AND NEURONS CONTAINING THE HYPOTHALAMUS.

K. Commins*, and T.A. Miller.

Electrophysiological studies have suggested that actions of opioids in the hippocampal formation are mediated by inhibition of GABA interneurons; however, an anatomical basis for this interaction has never been demonstrated. Thus, we sought to determine the ultrastructural relationship of leu-enkephalin inhibitory neurons containing terminals (GABAergic) and GABAergic neurons using double labeling immunohistochemistry. We found that in the hippocampal formation, GABAergic neurons contain terminals in the dentate gyrus, CA1, and CA3. These findings suggest that there are functionally different populations of GABAergic neurons in the hippocampus, and that the relationship between these populations may depend on the anatomical location of the target neurons.

KAPPA RECEPTOR mRNA LOCALIZATION IN THE GUINEA PIG BRAIN.

COMPARISON TO [H]IREMAMAGazine AND [H]JHRG-595 BINDING.

Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Three opioid receptor types have been identified in the CNS that are referred to as μ, δ, and κ. Given the recent cloning of the guinea pig κ receptor (Xue et al., Proc. Natl. Acad. Sci. USA, in press), this study examines the mRNA distribution of the κ receptor in the guinea pig brain and compares the mRNA patterns. The kappa receptor binding sites defined either by [H]JHRG-595 (43nM) or [H]iREMAMAGazine (30nM), in the presence of a 500 fold excess of DAMGO and DPDPE using in situ hybridization and receptor autoradiographic techniques. Kappa receptor mRNA was identified with a 5′-32P探针 probe generated by EcoRI-BamHI fragment of the guinea pig κ receptor (602-1334) that spans from extracellular loop 1 to near the C- terminal end of the receptor coding region. An excellent correspondence is observed between κ receptor binding sites and mRNA in the deep layers of cortex, caudate-putamen, nucleus accumbens, olfactory tubercle, dentate gyrus, and entorhinal cortex. Thus, observed, was counter in the region as the substantia nigra ventral tegmental area, where κ mRNA was limited to the substantia nigra, pars compacta and ventral tegmental area area, while κ binding is primarily in the pars reticulata. This suggests μ receptors may be synthesized in the striatum and transported to the substantia nigra, pars reticulata, or alternatively, may represent the binding sites for κ receptor antisense oligos. Similarly, κ receptors are synthesized in the substantia nigra and ventral tegmental area and transported to the striatum. Species comparisons to the κ mRNA data will be discussed.

ROLE OF BRAIN OPIOIDS IN THE DEVELOPMENT OF HYPERTENSION IN STRESSFUL BORDERLINE HYPERTENSIVE RATS.

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College of Pharmacy and TNR, University of Kentucky, KY 40506.

Opioid peptides have been implicated in modulating autonomic function under basal and stressful conditions, but their role in regulating cardiovascular function remains unclear. The borderline hypertensive (BHR) rat is a well-established model of neurogenic hypertension because its genetic background makes it susceptible to the hypertensive effect of stress. In this study, we examined the effect of a time limited period of restraint stress on the development of hypertension and the role of brain opioids on the development and maintenance of hypertension in BHR. We found that restraint stress, 2 hr daily, 5 days a week for 4 weeks, caused a significant rise in systolic blood pressure one week after beginning stress treatment. This increase in blood pressure (BP) continued throughout the period of stress (5 weeks). Moreover, restraint BHR continued to have higher blood pressure compared to the control group. This increase in systolic blood pressure was reversed by the administration of the μ-opioid receptor antagonist DAMGO (0.05nmol, iv) after the 4th week of restraint stress.

MULTIPLE EXPRESSION OF OPIATE TOLERANCE IN HYPOTHALAMIC ARCUTE NEURONS.

Department of Physiology, Oregon Health Sciences U., Portland, OR 97201.

To determine if hypothalamic arcuate (ARC) neurons develop tolerance to μ-opioids, intracerebroventricular recordings were made with bicucullin-filled electrodes in ARC neurons in hypothalamic slices from placebo and chronic morphine-treated (4 x 75 mg pellets for 2 d + 6 more pellets for a total of 6-8 d) or ovariectomized female guinea pigs. A dose-response curve was generated measuring the hyperpolarization in response to the selective μ-opioid agonist DAMGO. β-endorphin (bEND) neurons were identified using double labeling. Chronic morphine tolerance (EC50 = 245 ± 22 nM, N = 12; EC60 ± 33 nM in controls, N = 50) increased efficacy (ΔVmax = 7.1 ± 1.1 mV vs -10.7 ± 0.6 mV) and a 60% decrease in ΔGmax in all bEND neurons (N = 5) and other ARC neurons (N = 7). In another population of tolerant ARC neurons, DAMGO was less potent (EC50 = 110 ± 4 nM) without a change in efficacy. A third population of neurons (N = 6) did not exhibit any signs of tolerance with morphine treatment. Morphine-tolerant neurons also exhibited a higher incidence of k-opioid sensitivity in sensitized animals (20% vs 4%, p < 0.01). Therefore, chronic morphine uncouples μ-opioid receptors from k-opioid binding sites and increases k-opioid in ARC neurons. Since bEND neurons are dopaminergic neurons, similarly, μ-opioid receptors on the forebrain, this would not only affect neuropeptide but also homeostatic and reward circuits. (Supported by PHS grant DA05158)
OPIOIDS: ANATOMY AND PHYSIOLOGY III

T08.13

To determine how opioid receptor agonists affect the primary afferent neurotransmission, we examined the actions of dynorphin A(1-17) (DynA), (DPDPE), Tyr-D-Ala2-D-Leu5-enkephalin (DADLE) and the selective K-opioid agonist Dyn, (n = 6) and DynA (n = 11). When applied to the dorsal horn slices (1.75 x 1.5 x 0.8 mm), DynA produced a dose-dependent depression of the monosynaptic EPSPs (7.1 ± 0.6%, 9/21 days, 0.8 or 0.16 μM) that was reversed (by 97.3 ± 4.1% of control, n = 15, 6/21 cells) by naloxone, norbinaltorphimine, nor-binaltorphimine, or nor-binaltorphimine, respectively. Similar dual modulation of EPSPs was observed with DynA (0.5, 2.5, 10 μM) and DynA (10 μM) produced the same results in the presence of nor-binaltorphimine (0.1 μM, 5/5 cells). The results indicate that distinct modulation of synaptic efficacy can be induced in primary afferent synapses with neurons in the superficial laminae of the spinal cord. We speculate that dynorphin A(1-17) receptor activation and that these changes may be physiologically relevant for transmission and integration of sensory information, including pain. (Supported by NS-26323 and NIH-N29046EC.)

T08.14
DYNORPHIN A MODULATES EXCITABILITY IN AMPHIBIAN AND MAMMALIAN NERVES. M.R. Luna and M.F. Pacheco. Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Colima, Col. 28045 México.

The effects of dynorphin A 1-17 (DYN) on nerve excitability were studied in isolated sciatic nerves of the frog and rat. The amplitude of recorded diaphasic action potential was gradually reduced by increasing concentrations of locally applied DYN (10 - 100 μM). Amplitude inhibition attained, at each DYN concentration, was not modified by increasing stimulation frequency up to 10 Hz, however, higher stimulus reduced the degree of inhibition. DYN effect on action potential amplitude was concomitant with a decrease in conduction velocity and a 1 to 3 fold increase in threshold strength input-output curve, reaching maximum effects after 15 to 35 min of DYN application, and recovering completely (in 86% of the experiments) after 25 to 700 min of drug washout. DYN actions were antagonized by nor-binaltorphimine (50 μM), therefore, our data are indicative of a widespread action of DYN on nerve conduction, which results in an excitability decrease through a specific interaction with K1-opioid receptors. Finally, these results provide an additional mechanism in order to explain reversible falcid hindlimb paralysis and loss of reflexes, observed in rats, after intrathecal administration of DYN (Stewart & Isaac, Brain Res. 543:222-325, 1991).

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T08.15
INHIBITORY ACTIONS OF OPIOIDS ON SYNAPTIC TRANSMISSION IN THE RAT NEOSTRIATUM. B. Schlesser, M. Kundermann, B. Sutor, and G. ten Bruggencate. Department of Physiology, University of Munich, D-80336 Munich, Germany.

In the neostriatum, opioid receptors exist at a high concentration. Furthermore, enkephalin is colocalized with GABA in spiny stellate cells indicating that opioids may regulate the activity in striatal synaptic circuitries. In the present study, we investigated the influence of opiate-receptor activation on neuronal neurons in vitro using intracellular recordings. Experiments were performed on rat neostriatal slices (400 μm) with maintained corticostriatal connection. In the recording chamber the slices were kept submersed in artificial cerebrospinal fluid (ACSF). Synaptic activity was evoked by intracortical or intrastriatal electrical stimulation. Drugs were applied in addition to the ACSF. Application of the opioid receptor agonist D-Ala2-D-Leu5-enkephalin (DADLE) and the selective K-opioid agonist D-Pen2-7-Enkephalin (DPDPE) (0.1-1 μM) produced a dose-dependent decrease in the amplitude of both stratially or cortically evoked synaptic potentials. The opioids did not affect membrane potential or current-voltage relation. The effects were blocked by the opioid antagonist naloxone (1 μM) and persisted when GABA-ergic components were abolished by the GABA_A receptor antagonist bicuculline (20 μM). The data demonstrate, that activation of α-opioid-receptors decreases synaptic transmission in the neostriatum mainly by reducing glutamatergic excitation. (Supported by the BMFT, 01 K19001.)

T08.16

Neuropeptide FF (NPFF) is a FMRFamide-like peptide with opioid modulating activity. Neuropeptide FF (NPFF) is highly localized in the spinal cord where there are also specific NPFF receptors. Furthermore, there have been studies indicating that NPFF may participate in the regulation of pain threshold in the spinal cord. Thus, in this study, possible relationships between opiates and NPFF were investigated by studying the effects of various opioid receptor ligands on the K+-evoked release of NPFF immunoreactive material (IR) from rat spinal cords. Neuropeptide FF receptors were carried out by using an in vitro superfusion of isolated intact rat spinal cord. The opioid agonist U50488H decreased the K+-evoked release of NPFF in a dose dependent manner. In contrast, the µ-opioid agonist, Tyr-D-Ala-Gly-N-Me-Phe-Gly-ol (DAGO), was inactive and δ-opioid agonist, Tyr-D-Pen-Gly-D-Pen (DPDPE), exerted only a very slight inhibitory effect. The inhibitory effect of U50488H was antagonized by the K-opioid antagonist nor-binaltorphimine (NBI). NBI was found to enhance the K+-evoked release of NPFF probably resulting from the concomitant release of endogenous dynorphin by the K7. In view of the role of dynorphin in the antinociception, the results of this study further suggest that spinal cord NPFF participates in the regulation of opioid mediated antinociception.

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109.1 LACK OF NMDAR1 EXPRESSION IN LUTEINIZING HORMONE-RELEASING HORMONE NEURONS OF THE MALE SYRIAN HAMSTER. G. Muree, V. Giridant, S.G. Kohana and H.F. Urbanski. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

There is substantial evidence demonstrating that excitatory amino acid (EAA) receptors are involved in luteinizing hormone-releasing hormone (LHRH) secretion both in vitro and in vivo. Since immunolabeled LHRH neurons (G1 cells) that lack NMDAR1 gene it has been proposed that EAA's stimulate the secretory activity of LHRRH neurons directly. We have used immunocytochemistry with a specific antibody to NMDA receptor subunit 1 (NS1) in an attempt to confirm whether particular facilitatory influence of EAA's is mediated by such a direct neuronal pathway. Adult male Syrian hamsters were killed with heparinized saline injected under ether anesthesia and their brains fixed with 4% paraformaldehyde. Coronal Vibratome sections (50 μm) containing the medial preoptic area were then processed initially for immunocytochemistry using a monoclonal antibody to LHRH (H14H) and stained with diaminobenzidine. Subsequent NS1 (for NMDAR1 mRNA using a 32-p end-label to apply to probes) The DNA template was extracted from NMDAR1 cDNA using Pest and EcoRI was performed on these same sections and counted G1 cells. After dropping in photographic emulsion, silver grain deposition was examined microscopically to determine colocalization of NMDAR1 mRNA within the immunostained LHRH neurons. As expected, G1 cells clearly demonstrated hybridization of the riboprobe. In the brain sections, a high density of silver grains was localized in the outer fringe of the preoptic area and within the hippocampus but not in the vicinity of the LHRH perikarya; only 1 neuron from a total of 103 neurons/3 animals showed possible colocalization. These results suggest that although G1 cells may have an inherent capacity to express the NMDA receptor, in the in vivo excitation of LHRRH neurons by EAA's is more likely to be mediated indirectly, by a pathway that involves interneurons.

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In the male quail forebrain, aromatase-immunoreactive (ARO-ir) neurons are clustered within the sexually dimorphic medial preoptic nucleus (POM), the nucleus accumbens septi (NDST), the median eminence (ME), and the hypothalamic nucleus suprachiasmaticus (SSC)(1). ARO-ir neurones are also present in the lateral hypothalamus. Previous studies demonstrated that ARO cells are sensitive to testosterone (T) and are involved in the control of gonadal steroid function after castration and administration of these steroids. In this recent study, we demonstrated that the vasotocinergic system is also sensitive to T: the density of the vasotocin- (VT-ir) fiber networks located within the POM and the lateral septum (LS) was higher in castrated compared to gonadectomized male birds and restored to intact level by a treatment with T. We analyzed here the anatomical relationship between ARO and VT systems within these regions. The study was performed on brains perfused with Zamboons fixative and serially sectioned with a cryostat. Sequential staining for VT, ARO, or VT-ARO was performed on adjacent 30 μm thick sections. High concentration of VT-fibers were observed within the POM, the LS, the lateral septum, and the ventral hippocampus. In double-labeled sections, all clusters of ARO-ir cells, with the exception of those localized in the Ac, were embedded in a dense network of VT-fibers. Many of the VT-ir terminals appeared to end in the neuropeptide surrounding ARO-ir elements rather than directly on their cell bodies. However, there was a very close correspondence between the extension of the ARO-ir cells and VT-fibers. This study, therefore, suggests the role of the T-dependent ARO system is also directly innervated by a T-dependent peptideergic system which can be modulated by steroids both directly and indirectly through the VT system. Further studies will be needed to determine whether this VT interaction has a functional significance for the ARO-ir cells and whether these two neurochemical systems (ARO and VT) affect reproduction in an independent or coordinated manner. Supported by grants of ESF, COR, MCSR1, FRFC, P.A. is a fellow of the Minister of pi. Gi. Este.

109.3 FOS EXPRESSION IN GNRH NEURONS WITHIN PREGNANT AREA (POA) GRAINS OF THE POSTNATAL HYPOTHALAMUS (HPG) MICE: EFFECT OF PROGESTERONE AND SEXUAL BEHAVIOR. M. G. O'Connor, J. T. J. Wang, O. M. Miller, and A. J. Silverman, Dept. of Medicine, Mount Sinai School of Medicine, NY 10029 and Dept. of Anatomy, Columbia University, Columbia, NY 10032.

Expression of Fos, the proto-oncogene product of the c-fos gene, is widely accepted as a marker for neuronal activation. We have shown that in normal steroid-primed, ovulating estrus mice, Fos expression occurs in greater than 40% of GnRH neurons and is maintained at this level for a longer period of time in similar treated females paired with an uncastrated male (Emde and Simerly 1988). Postpartum, GnRH neurons our functional GnRH gene and are immature. After receiving intravaginal POA grafts containing GnRH neurons, females may enter persistent estrus, and have the capacity to ovulate reflexively or, more rarely, ovulate in response to a preoptic stimulus. The present study of HPGPOA mice examined the effects of both progesterone treatment (P; 500 μg 0910h) and sexual behavior (introduced to an experienced male at 1400 h) on the expression of Fos in grafted GnRH neurons. In response to sexual behavior, 47±9.10% of GnRH neurons of P-treated HPGOPOA mice expressed Fos in contrast to 9.5±0.96% of GnRH neurons in those mice that did not receive P. Treatment alone did not increase the expression of GnRH neurons (4.0±0.4%). These results suggest that in the presence of P, sexual behavior may serve as a stimulator of Fos expression in GnRH neurons. In addition, the present study supports the hypothesis that at least a portion of the graft-host connectivity that underlies aspects of reproductive competence in HPGPOA mice is steroid sensitive.

Supported by NIH HD 19077.


The hypotalamic arcuate nucleus contains a neuronal population immunoreactive (ir) for proopiomelanocortin (POMC) and corticotropin-β (POMC-β) derived peptides and giving a sexually differentiated projection to the median eminence: to the subependymal (S) region and to the gonadotrophs (G) in the rich regions of the posterior hypothalamus (Ph). The presence of the POMC-β ir in males and females and in female rats. The presence of the POMC-β ir in males and females and in female rats. The presence of the POMC-β ir in males and females and in female rats.

The male median eminence is sensitive to changes in the arcuate nucleus of cell bodies and is protein for neurotensin (NTX). In the female rat, the arcuate nucleus of cell bodies is sensitive to NTX and the functional significance of this is the neurotensin (NTX) system in the male rat.

To study this point, results suggest a postnatal androgen-driven "non numerical" mechanism of differentiation for this neuronal system of the mammalian hypothalamus whose organization involves development of the autonomic system.

Supported by ROI MH39453 and 15SHOP33283.

109.6 EFFECTS OF INTRAUTERINE POSITION ON METABOLIC CAPACITY OF THE HYPOTHALAMUS. G. S. M. R. C. M. H. M. S. M. H. O. D. G. O. H. and J. C. Clark. Dept. of Psych and Zoology, Univ of Texas, Austin TX, 78712 USA and Dept of Psych, McMaster Univer, Ontario L8S 4K1, Canada.

Neural metabolic correlates of known effects of intrauterine position on behavior in the Mongolian gerbil illustrate the ability to investigate morphological determinants. Hypothalamic regions were compared in female gerbils that developed in utero between two female fetuses (2F) or between two male fetuses (2M) to assess differences in metabolic capacity. A total of 25 female rats were studied. A quantitative image analysis systems we measured the cytochrome oxidase (COX) activity in histochimically stained brain slices of the gerbil. Due to its limiting factor in oxidative metabolism (O) is determined by an excellent marker of metabolic capacity: increased activity of neurons in a brain region lead to increased C.O. content in their mitochondria. The COX staining procedure involved metal impregnation through cobalt preincubation, as well as oxygenation and heating of the reaction medium. Optical density (O.D.) of ten hypothalamic regions, including the sexually dimorphic anterior hypothalamic area (AHA), the orbital cortex and the grey matter O.D. Significant group differences in the metabolic capacity of three hypothalamic regions were revealed. Differences in the AHA in the anterior hypothalamic area and the sexually dimorphic area were seen in 2M as compared to 2F. To the best of our knowledge, this is the first examination of functional changes in hypothalamic areas related to the differences in intraterine position. Supported by RO1 MH43353 and 15GSP037338.
709.7 THE DISTRIBUTION OF AROMATASE mRNA IN THE BRAIN OF ADULT MALE AND FEMALE RATS USING in situ HYBRIDIZATION C.K. Wagner* and J.J. Morrell, Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102

Many of the effects of gonadal steroid hormones in the male brain are due to the actions of the testosterone metabolite, estradiol, which is synthesized by the actions of the P450 aromatase enzyme, aromatase. Aromatase activity has been demonstrated through the use of in vitro enzyme assays, to be present in regions of the preoptic area, hypothalamus and limbic system. Levels of aromatase activity are much higher in male than females in some brain regions. The present study examined the distribution and cellular localization of aromatase mRNA in adult male and female rat brains using in situ hybridization. A 113-base 5’-labelled cDNA probe, complementary to the aromatase mRNA just upstream from the heme-binding domain, was synthesized from the rat aromatase cDNA inserted into pGEM2 (generous gift of Dr. Richard O’Riordan, Houston TX), using a Bal31 fragment and SP6 polymerase. Following the hybridization procedure, sections were dipped in nuclear track emulsion to order to detect aromatase mRNA at the cellular level. In the male, many heavily labelled cells were found in the unoccupied bed nucleus of the stria terminalis and the medial amygdala. A smaller number of heavily labelled cells were seen in the periventricular preoptic nucleus, the median preoptic nucleus, the ventromedial nucleus, the cortical amygdala and the sublenticular organ. Lightly to moderately labelled cells were observed in the horizontal diagonal band, the magnocellular preoptic nucleus, the median preoptic nucleus, the anterior hypothalamus, the arcuate nucleus, the piriform cortex and nuclei of the thalamus.

Aromatase mRNA was found in the same brain regions in females. Sex differences and the effect of castration in males on levels of aromatase mRNA will be discussed.

Supported by HD 22588 to JIM and NBA 47 0974 to CWJ and JIM.


Immunohistochemical studies localizing the calcium-binding proteins (CaBPs) parvalbumin (PV), calbindin (CB), and calcitelin (Ck) in the monkey and the primate visual areas V1 and V2 and have shown that these CaBPs are present within morphologically distinct groups of neurons that display specific regional and laminar distribution patterns. In order to further characterize the cellular organization of the primate visual system, we performed a quantitative analysis of the distribution of CaBPs-immunoreactive (ir) neurons in different divisions of the magnocellular (M) and parvocellular (P) visual pathways in the macaque monkey. Area V1 was characterized by very high numbers of PV- and CB-ir neurons, while Ck-ir neurons were less numerous. Areas V2, V3 and V3A displayed lower numbers than area V1 for the three neuronal populations. However, cell counts markedly increased in areas at higher levels in the cortical hierarchy. In particular, areas MT, VIP, V4 and TE showed very high densities of stained neurons. In addition, a subset of CB-ir pyramidal neurons located in layer III exhibited distinct distribution patterns among the cortical areas investigated, with the highest densities in areas V4 and MT and slightly lower cell counts in areas TE, TE, VIP and LIP. These results indicate that CaBPs define neuronal populations that are differentially represented among cortical regions of known function in the M and P pathways in the primate visual system. (Supported by NIH AG06647, MH52154 and the Brookdale Foundation)


Prior physiological studies of subjective contours used patterns which induce bars or edges by line ends or corners (van der Heijdt et al., Science 224:1290-1294, 1984). By contrast, we employed a more complex figure in which a Kanizsa square is induced and placed in apparent motion. The Kanizsa square moves as if both along a double row of inducers ("PAC-MAN"). This illusion is achieved by appropriately orienting neighboring inducers in successive planes and randomly orienting inducers elsewhere (Bravo et al., Vision Res. 28:881, 1988). At eccentricities equal to those tested, six observers perceived the illusion as a black square, black-occupied object moving against the background. If the open quadrants of the inducers were closed by thin circular arcs, no such object was seen (control stimulus). We recorded from cells in areas V2 and V4 of one feasting monkeys and sought a cellular correlate of the perceived illusory object. We attempted to place all inducing elements outside the classical receptive field. In V2, 659 cells (eccentricities 3.4-13 degrees) responded to the moving Kanizsa square but gave a reduced response or no response at all to the control stimulus. In preliminary data, 391 cells (eccentricities 1.5-15 deg.), 413 cells showed similar behavior. Response peaks correlated with the position of the induced object centered on the receptive field. Some cells were driven by a double row of inducers selectively tuned to patterns in which inducers and background colors were photometrically isoluminant. In some cells, area V4 inducers (to be a control color stimulus) abolished the subjective square response. In another case, increasing luminance contrast to 10% but removing background and inducing achromatic did not evoke an increased response.

These data confirm the presence of illusory contour responses in V2 and extend these findings to a more complex stimulus. In addition, we have preliminary evidence for similar responses in V4. Our results suggest the existence of figural completion mechanisms for chromatic patterns similar to the boundary completion mechanisms shown earlier for achromatic patterns.

710.4 MECHANISMS OF CONTOUR PROCESSING IN MONKEY PREBRAIN CORTEX INCLUDE INFORMATION ON DIRECTION OF GRAVITY. X. M. Saam, V. Henn, and E. Peterhans (SPON: European Neuroscience Association). Dept. of Neurology, University Hospital, Zurich, Switzerland, CH-8091 Zurich, Switzerland.

Form perception requires a generalization of representation of contours with regard to the quality defining them (e.g. luminance, color, motion, or texture) and their spatial position and orientation. We report here the effect of body tilt on mechanisms of contour processing in the visual cortex of the alert alert monkey.

The responses of single neurons were studied while the monkey performed a visual fixation task in either an upright position, or with its body tilted about the naso-cranial axis by 25° or 50° (recording in 10° increments). A total of 106 orientation selective neurons were both in striate and prefrontal cortex (areas V2 and V3a/V4A).

We found that the position of the response field for light and dark bars or as at 2 or 3 body positions. In striate cortex, most neurons (94%) were of a non-compensatory type showing altered orientation and position of the response field according to the body tilt and the estimated counterrolling of the eye. In parietal cortex (32±13.4°) by 25°. By contrast, 40% of the neurons in prefrontal cortex (82%) were of a compensatory type. These cells, although showing a change in field position, preferred similar stimulus orientations at all body tilts. This indicates that the orientation sensitivity of these neurons was invariant with respect to the direction of gravity.

In conclusion, information about the direction of gravity is implemented in monkey visual cortex first at the level of area V2. This suggests that otolithic signals contribute to mechanisms of contour processing at early stages of visual processing.

Supported by ESFR BII 6019 and Mucron II 6615.
110.5 TOPOGRAPHIC PATTERN OF CORTICAL CONNECTIONS TO DORSAL AND VENTRAL VISUAL AREAS IN MACAQUE MONKEYS. J. Steriade, G. Fite, and E. Schiller. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

We studied the connections of V2 with other extrastriate visual areas in macaque monkeys (Macaca mulatta) to better determine their extent and visuotopic organization. Multiple injections of fast blue and fast green fluorescent tracers and WGA-HRP were made in dorsal V2 representing the lower central vision and in ventral V2, representing the upper central vision. After 4-7 days survival, animals were anesthetized and perfused, and visual cortex separated from the rest of the brain. Cortex was manually flattened and cut perpendicular to the surface. Toed labels were related to retinotopic boundaries identified from adjacent primary and extrastriate visual cortex. It was observed that these areas project to two main visual areas. After further, intracellular recordings, the specificity of projections originating from V2 stripes. For instance, the superior colliculus (SC) receives a strong projection from V2, and available information indicates that the connections in the monkey are predominantly magnocellular in nature. We have therefore correlated the distribution of V2-SC projection neurons with the array of CO V2 stripes.

110.6 THE DISTRIBUTION OF CORTICO-COLICULAR PROJECTION NEURONS CORRELATES WITH THICK CYTOCHROMOGENIC AXON PROJECTIONS IN VISUAL AREA V2 OF MACAQUE MONKEYS. J. Steriade, G. Fite, and E. Schiller. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

Several lines of evidence suggest that the three classes of CO stripes observed in V2 (CO-dense thick axons, sparse thin axons, and pale intercalated stripes) are related to specialized functional streams in visual cortex. One line of evidence comes from the pattern of connections that CO stripes and visuotopic areas. Thus, in accordance with the idea that thin CO stripes are part of the magnocellular processing stream, these stripes have been shown to project to areas involved in the analysis of motion and spatial attributes, such as area MT. Exploring the pattern of V2 connections with subcortical nuclei that have magnocellular functions confirmed the specificity of projections originating from V2 stripes. For instance, the superior colliculus (SC) receives a strong projection from V2, and available information indicates that the connections in the monkey are predominantly magnocellular in nature. We have therefore correlated the distribution of V2-SC projection neurons with the array of CO V2 stripes.

Guided by readings of visually evoked potentials, multiple injections of either HRP or fluorescent tracers were made into the SC of adult monkeys (Macaca fascicularis). Three- to four-day later, the area was sectioned coronally or tangentially to the cortical surface, and the distribution of labeled cells in layer V was compared to the CO staining pattern. In each area, we observed the specificity of projections originating from V2 stripes. For instance, the superior colliculus (SC) receives a strong projection from V2, and available information indicates that the connections in the monkey are predominantly magnocellular in nature. We have therefore correlated the distribution of V2-SC projection neurons with the array of CO V2 stripes.


Extrastriate area V3 may represent an important site for integration of visual information inputs from both the retina and parvocellular pathways and has prominent projections to both areas MT and V4. Therefore, we investigated the representations of color and motion in V3. We recorded from single cells in area V3 of macaque monkeys (Macaca mulatta) using standard acute recording techniques. After measuring each cell's spatial and temporal properties, we tested its chromatic and motion sensitivity using overlapping arrays of moving and static stimuli. All V3 cells were orientation selective. About 50% of our sample was selective for direction of motion. Contrary to previous reports, we also found that about 50% of the remaining cells were color selective for color. These two substrategies overlapped to a large extent, and we found a significant proportion of cells highly selective for both of these stimuli. An analysis of the responses of directionally selective cells to moving stimuli showed that in area V3, as in MT and unlike in V1, V2, and V4, many cells were not selective for the orientation of the plaid pattern, rather than the component motion. Furthermore, a third of our sample was reshaped, and many of these cells showed orientation or direction-selective surround effects. Our results show that there is a significant interaction between color and motion processing in area V3, and that V3 cells possess many of the complex motion properties typically observed at later stages of visual processing. Supported by MRC G920389IN and NIH EY01001.

110.8 RECEPTIVE FIELD STRUCTURE OF V2 NEURONS IN THE PRIMATE PRIMATE GALAGO CRASSIGAMAUDUS. J. D. Allman and V. A. Casagrande. Dept. of Cell Biology, Vanderbilt University, Nashville, TN 37232-2175.

Visual area V2 (V2) is the second largest visual area in the primate cortex and receives input from all other extrastriate areas. There is very little information about the receptive field (RF) structure of V2 cells. A key question is how the RF structure of V2 cells compares to the RF structure of V1 cells. To answer this question we measured the response of simple cells in three different primate species using standard single-unit electrophysiological techniques with both bars and drifting sine-wave gratings. All cells were classified as simple or complex and their RFs were characterized by orientation and spatial tuning and contrast sensitivity. We found that complex cells, especially those responsive to grating structure, were more sensitive to orientation selectivity and also more contrast sensitive than simple cells. The majority (71%) of these cells were binocular and directionally selective (78%). The average orientation tuning was 21.5 ± 9.65° SE1. The preferred spatial frequency averaged 0.5 cycles/deg with a mean tuning bandwidth of 2.4 ± 0.68 SE octaves and a mean cut-off of 1.2 cycles/deg. The mean preferred temporal frequency was 0.276 ± 1.85° SE Hz with a cutoff frequency of 8 Hz. The average contrast sensitivity was 20.1 ± 19.32 SE. The RF structure of V2 cells near area centrals was very similar to that of V1 cells in this species. V2 cells differed from V1 cells in having larger receptive fields, being mostly binocular, and having lower spatial frequency peaks and cutoffs.

Supported by EY06410, EY07178, EY08126, HD15602.


Using anterograde techniques, we have found that corticotectal (CT) neurons in primary visual cortex (area 17, n = 21) and a lateral visual cortical area (posteriormedial suprasylvian visual cortex, PMLS, n = 13) in cats trained in ocular dominance tasks. Head position was fixed, and gaze was monitored using the scleral search coil technique. The cat faced a rear projection screen onto which we could project a visual stimulus. CT cells in both areas responded well to small stimuli moving away from the target when the target was in the receptive field. None of the cells in either cortical area exhibited pre-saccadic activity. At least half the CT cells in area 17 showed post-saccadic activity associated with visually guided saccades. The retina across an illuminated field. PMLS CT cells did not show post- saccadic activity. Furthermore, both CT cells in PMLS 17 respond to visual stimuli well in excess of saccade velocities, an ocular motor signal must actively suppress visual inputs when the cat makes eye movements. In general, area 17 CT cells are like their counterparts in the paralyzed, anesthetized preparation. Most are strongly directional, orientation selective and show little evidence of length summation. PMLS CT cells are the same in both species. This is probably because the cells make decisions about which target to look at next in contrast, the large receptive fields and directional anisotropy of PMLS CT cells would be consistent with a role in supporting visually-guided locomotion (Supported by NIH Grant EY06818).

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
THALAMIC AND CORTICAL CONNECTIONS OF AREA 18 OF ALBINO CATS. Marie Peschel* 1, Andrei Wrob, Iwona Stojennicka and Jan Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

The visual system of albino animals are abnormally in that many ganglion cells are mislocated to the contralateral geniculate nucleus (LGN). The consequences of this misrouting are only partially understood. We studied the organization of albino geniculocortical and corticogeniculal connections by injecting up to 6 different tracers in distinct rostrocaudal locations in area 18 (V2) of 4 animals. The results revealed a roughly normal rostrocaudal sequence of the geniculate projections and progressively more rostral injections in area 18 labeled progressively more rostral locations in the LGN. However, the zones of labeled cells were larger than those for comparable injections in normal cats suggesting greater divergence and convergence of LGN projections. Layer A1, with abnormal retinal inputs, had discontinuous medial and lateral foli of labeled neurons from single injections, indicating topographic mismatching in projections. Cortical connections with areas 17 and 19 and suprasylvian regions were also roughly normal with more rostral injections labeling more lateral zones in these fields. However, again connections appeared less precise than normal with connections of central area 18 in particular being more widespread and overlapping than in normal cats. These abnormalities suggest that in albino cats, visuo-tactile organization is less precise and orderly. Supported by EY-02666 (JK), NSPB-FFS P92037 (MP) & James S. McDonnell Foundation (AW).


We have previously shown that the cells projecting from area 17 of the cat to the parietal medial lateral occipital area (PMLS) are clustered within regions that stain densely for cytochrome oxidase (CO). Here, we extend these findings to connections involving area 18. Large, multiple injections of wheat germ agglutinin-conjugated horseradish peroxidase or cholera toxin into callosal target areas were made into areas 17, 18, or the PMLS. In each case, the aim was to saturate a large part of the visual field representation of the injected area. Labeled cells were charted in tangential sections of cortex and compared to the pattern of CO staining in alternate sections. After tracing in area 17, all labeled cells in area 18 were, as was previously found in area 17, arranged in patches that aligned with the CO blobs. In addition, the PMLS projecting patches, were slightly larger in area 18 (slightly greater than inmm spacing) than in area 17 (slightly greater than 1mm spacing). In agreement with Ferrer et al. (1988), we found the projection from area 17 to area 18 to be discontinuous. Moreover, cells projecting from area 17 to area 18 are confined to the CO blobs. Surprisingly, labeled cells and terminals in area 18, following large injections in area 17, also have a patchy organization, which we are currently trying to relate to the pattern of CO activity. Preliminary evidence suggests that they too are confined to the blobs.

Thus, while area 17 and 18 have similar PMLS-projecting blob and non-PMLS-projecting interbop blob compartments, it appears that connections between these two areas are made spacially and to the CO blobs, with interblop being unconcerned cortico-cortically. These results are not easy to interpret in a strictly hierarchical framework: classifying connections from 17 to 18 as feedforward, connections from 18 to 17 as feedback; rather, the similarities in connectional organization are consistent with suggestions that these two areas operate in parallel. (This work funded by MRC 5-99150)

TWO VISCERAL SYSTEMS IN A RODENT. C.Q. Elliott* and M. Dias, Dept. of Psychology, University of Waterloo, Waterloo, Ontario, Canada.

Although it is sometimes assumed that there are two visual cortical processing streams in non-primates, there is little evidence for such a disjunction of function in rodents. We wanted to demonstrate selective efficacies to either dorsal or ventral cortical areas on two different types of visual information processing in the Mongolian gerbil. Gerbils received either lesions of parietal cortex, ventral cortex, or sham surgery. After recovery, they were placed at random starting locations into an open field containing an object for one five-minute trials. On each trial, the total time spent in contact with the object was measured. On each of the first 5 trials, the object was moved to a new location in the field and a new, different object was placed in the old location. The critical measure was the amount of time spent in contact with each of the objects on each of the first 5 trials. Normal gerbils showed decreasing contact time with the object over the first five trials. On the sixth trial, they showed elevated contact time with both the new object and the object at a new location, demonstrating sensitivity to both object location and identity. Gerbils with parietal lesions showed relative disorientation to the new object but not the new location, suggesting a deficit in object recognition. On the sixth trial, the object was moved to a new location in the field and a new, different object was placed in the old location. The critical measure was the amount of time spent in contact with each of the objects on each of the first 5 trials. Normal gerbils showed decreasing contact time with the object over the first five trials. On the sixth trial, they showed elevated contact time with both the new object and the object at a new location, demonstrating sensitivity to both object location and identity. Gerbils with parietal lesions showed relative disorientation to the new object but not the new location, suggesting a deficit in object recognition. These findings constitute evidence that lesions to ventral cortical areas in rodents can produce dissociable effects on visual behaviour reminiscent of those seen in primates. (Supported by a N.S.E.R.C.C. grant to CGE).


We recently found that V2-A1 projections to the PMLS are confined to the CO blobs, suggesting that for area 18, 19, area 17, and 18 have segregated output areas for each of these regions. In the present study, we examined V1-A1 and V2-A1 connections to the PMLS in normal animals. In the presence of normal retinal inputs, all injections of cholera toxin or wheat germ agglutinin-peroxidase into the V1-A1 projection area were restricted to the CO blobs. In our previous study, these injections were into area 17. In this study, we have injected into area 18, the PMLS, and the lateral suprasylvian area (LSA). We find injections of cholera toxin conjugated to colloidal gold (CTB-AU) into the PMLS projecting area of area 18 (slightly greater than inmm spacing) than in area 17 (slightly greater than 1mm spacing). In agreement with Ferrer et al. (1988, we found the projection from area 17 to area 18 to be discontinuous. Moreover, cells projecting from area 17 to area 18 are confined to the CO blobs. Surprisingly, labeled cells and terminals in area 18 following large injections in area 17, also have a patchy organization, which we are currently trying to relate to the pattern of CO activity. Preliminary evidence suggests that they too are confined to the blobs. Thus, while area 17 and 18 have similar PMLS-projecting blob and non-PMLS-projecting interbop blob compartments, it appears that connections between these two areas are made specifically to the CO blobs, with interblop being unconnected cortico-cortically. These results are not easy to interpret in a strictly hierarchical framework: classifying connections from 17 to 18 as feedforward, connections from 18 to 17 as feedback; rather, the similarities in connectional organization are consistent with suggestions that these two areas operate in parallel. (This work funded by MRC 5-99150)


The modifiability of cortical cells was studied in visual primary (VI), nonprimary (SSS, PMLS, ALIS, and MD) and posterior areas of normal (MS or area 7) areas. Extracellular unit recording was made in 7 cats monocularly deprived (MD) at ages of 1-11 weeks and in 31 normal (NOR) controls. They were anesthetized and paralyzed, and the receptive fields (RFs) mapped and PSTHs analyzed. While similar responsiveness was found in VI cortical area, the total number of cells, responsive cells, and binocular cells were all higher in NOR than in MD cats. There was a trend for the MD cats to respond to the RFs of the SSS and MS areas of the MDs. These reports are consistent with the findings in the SSS areas of the MDs (see table). The binocularity is consistently reduced in all areas of MDs but more vigorously in the SSS and MS. This is in accordance with our previous data on selectivity of the cells' reaction and their RFs in MDs, outside VI (Sci. Neurosci. Abstr., 18:1315, 1992). There is, thus, an internal deficiency in processing visual information in areas not exclusively dedicated to vision, which is enhanced by the partial elimination of visual exposure during development. Supported by Adams Super Center for Brain Studies, Tel-Aviv Univ.
CEREBELLUM: GENETICALLY NEEDED RESPONSES TO MUSCLE OUTPUTS

T11.1


Vestibulocerebellar (VS) and cervicocerebellar (CS) reflexes show different patterns of postural adjustments depending on the direction of neck displacements. This organization may require the assistance of the cerebellar anterior vermis, which integrates both the vestibular and neck input to influence the motor output. A prerequisite for such a proposed function is that the vermis encode the spatial coordinates of both head and neck displacements.

We investigated, in decerebrate cats, the directional selectivity of the responses of visual Purkinje (P) cells to wobble either of the whole animal at 0.15 Hz, 10° (vestibular input) or of the body over a fixed head at 0.15 Hz, 2.5° (neck input). In both instances the direction of animal or body rotation moved at a constant speed over 360° in a clockwise (CW) or counterclockwise (CCW) direction. Bidirectional responses were represented by a vector lying midway between the maximal response directions to CW and CCW. These vectors were distributed over the entire plane of rotation for both vestibular (n=44) and neck responses (n=98), with some predominance of neck vectors in the first quadrant.

In conclusion, the cerebellar anterior vermis provides a framework for spatial coding of vestibular and neck signals and may represent the control of VS and CS reflexes during a variety of head and neck displacements.

T11.3


The % of cells responding to 70 dB clicks and hisses differed before: 8% in L VNT, 50% in flocculus, 49% in lat. anisiform lobe, and 37% in med. anisiform lobe. After conditioning, responses to CS were greater than to DS in the med. anisiform area, and exceeded peak responses to the CS before conditioning. The ratio of CS:DS responsive cells increased and reversed from that for adaptation and sensitisation. Separation of data from L V and VI also found responses after conditioning, at L V and VI. Discriminative initiation of the blink CR appears to depend on motor cortex and dorsal cochlear nucleus than on cerebellum even though contributions from the cerebellum may be needed for late components of the CR to develop and be performed. (Supported by HD05958.)

T11.5

SELECTIVE ELIMINATION OF CEREBELLAR OUTPUT IN THE GENETICALLY DYSTONIC RAT. M.S. LaDue*, J.F. Loden, and A. Meinzen-Derr. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, Alabama 35294.

The genetically dystonic (DY) rat, an autosomal recessive mutant, exhibits a progressive motor syndrome that resembles the generalized idiopathic dystonia seen in humans. Even with supportive measures, rats die by postnatal day 69. A total cerebellarectomy that includes the dorsal portion of the lateral vestibular nucleus (LVN) eliminates the dystonic motor syndrome of the dy rats, greatly improves overall motor function and prevents early death. After total cerebellectomy, dy rats survive into adulthood, mate and successfully rear their offspring. Selective elimination of cerebellar output was performed on 15-day-old dy rats in order to determine the cerebellar components critical to the mutant's motor syndrome. Electrolytic and/or excitatory amino acid lesions of the medial cerebellar nucleus, interpositus cerebellar nucleus, lateral cerebellar nucleus and LVN were created in separate groups of dy rats. Rats were observed for the presence of abnormal motor signs (falls, twitches, clamps, pivots) and tested on several measures of motor performance (activity, climbing, righting, homing) before surgery and again on Day 20. All nuclear lesions produced significant (p < .05) improvements in motor function and decreases in the frequency of abnormal motor signs. No individual nuclear structure appears entirely responsible for the dy rat motor syndrome. (Supported by NIMH-DNB MD15593-01, Dyslexia Research Foundation and American Association of Neurological Surgeons)

T11.6


Adult meandertail mutant mice (mea/mea), unclefected heterozygous mice (mea+), and wild-type mice (C57Bl) were euthanized, transcardially perfused with 4% formaldehyde, stained with Nissl, and embedded for routine methods. All forms of Purkinje cell were preserved, with an 80% increase in Purkinje cell density. A thin superficial layer consists of neuropil composed primarily of Purkinje cell dendritic profiles. Disorganized Purkinje cell somata with interposed neuropil form an intermediate layer. The deepest layer consists of glial and small neuronal somata, and a neuropil containing myelinolized fibers. Purkinje cell dendrites, and a few myelin fiber bundles. Scattered within the agranular cortex are micronuclei, a few of which appear to be ectopic granules. The neuropil of the agranular cortex contains large numbers of premaged Purkinje cell dendritic spines which are wrapped in glial lamellae, and are apparently not contacted by presynaptic elements. Presumptive basket cell axons are observed to impinge upon the surface of Purkinje cell somata and dendrites, and boutons with the characteristics of climbing fibers contact some presynaptic appendages. The cortex of the cerebellar posterior lobe is relatively normal in appearance. This "normal" cortex, however, exhibits a high frequency of ectopic granule cells located singly, and in clusters, at the surface of and within the molecular layer. An intermediate cortical region forms a gradual transition from the normal to the agranular cortical architecture. Supported in part by NHI grant NS22909 to LEM.

Lower thoracic-upper lumbar (LT-UL) spinocebellar (SpCb) projections have a complex topography in the anterior lobe of normal rats. In adult shaker rat mutants, many or all of the Purkinje cells (PCs) in the anterior lobe degenerate. This study sought to determine if loss of PCs alters LT-UL SpCb topography in the anterior lobe. In shaker rats the distribution of WGA-HRP labeled LT-UL SpCb mossy fiber terminals was compared to the distribution of the anterior lobe and compared with similar reconstructions from normal rats. In shakers, labeled LT-UL labeled terminals were localized to clusters and sharply defined, irregularly-shaped patches surrounded by terminal free areas. The terminal patches were contained within discontinuous parasagittally oriented stripes or transversely oriented bands. The spatial distribution of LT-UL SpCb projections in shaker rats have numerous features of organization similar to that seen in normal rats. Based on these similarities, we conclude that PCs are not necessary for the maintenance of SpCb topography in the rat cerebellum. (Supported by NIH Grants RO7013 and NS20227).


The role of the complex spike as a 'meaningful' afferent input for the cerebellar nuclei is much debated. Here, we study the response of identified neurons in the cerebellum to electrical simulation of the inferior olive. Ketamine anesthetized male Wistar rats received stimulation electrodes in the red nucleus and inferior olive. Recordings of neurons in the contralateral cerebellar nuclei and cerebellar cortex were obtained with glass microelectrodes filled with 4M NaCl. Cerebellar nuclear units were identified by their antidromic action potential triggered from the red nucleus which collided with spontaneous spikes. Purkinje cells were identified by their complex spike activity. After identification, the response pattern to graded inferior olive stimulation was recorded and analyzed. Seventy-five cerebellar nuclear neurons were analyzed. Besides a single orthodromically induced action potential in 44 units (presumably triggered by climbing fiber collaterals; latency 4.2 ± 0.4 msec, virtually all units (73) responded with an initial pause of variable duration (mean 57 ± 38 msec). Post-stimulus time histograms revealed that this inhibition phase was followed by an increase in activity in 61 units. For 36 units, the chance of firing more than doubled for at least 20 and up to 500 msec (mean 125 msec). Graded stimulation revealed that, frequently, the inhibition developed before the facilitation. Purkinje cell recordings indicated that it is unlikely that the facilitatory effect upon cerebellar nuclear neurons is exclusively due to a change in simple spike firing rate. It is concluded that olivary triggered complex spikes not only cause a phase inhibition of cerebellar nuclear neurons but, in addition, may result in a subsequent, and potentially significant, increase of their firing rate. This effect appears to be especially prominent when large members of olivary neurons fire in synchrony.


Spike responses were investigated in a slice preparation of the turtle (Trachemys scripta elegans) cerebellum by current clamp whole-cell recordings. IPSPs, presumably due to Golgi cell activation, were evoked by electrical stimulation in the granule cell layer and in the molecular layer. Three types of inhibitory responses were found: the classical bicuculline and picrotoxin sensitive response with reversal potential near the Cl⁻ equilibrium potential. This IPSP had a relatively short latency, lasted around 100 ms and strongly reduced the amplitude and duration of the following EPSP. In some cells a part of this IPSP persisted following the addition of bicuculline (50 µM) and picrotoxin (50 µM). Under these conditions glycine (focal application or 200 µM bath-applied) evoked a strychnine-sensitive (50 µM) response with a reversal potential close to the Cl⁻ equilibrium potential. The bicuculline and picrotoxin-resistant glycine response is consistent with the expression in granule cells of glycine-receptor β subunits, insensitive to picrotoxin (Lun and Bockfeider, 1991). These cells showed a slow IPSP, a slow IPSP, developed with a maximum after 500-1000 ms, and lasting some seconds. This IPSP had a reversal potential close to the K⁺ equilibrium potential and was blocked by CgP 35348 (200 µM), consistent with a GABA A response. This IPSP was particularly prominent in the presence of bicuculline and picrotoxin: under these conditions microiontophoresis of glycine displayed amplitude depression, correlating in timecourse to the GABA A IPSP. Furthermore, the mossy-fiber paired-pulse depression was strongly reduced by CgP 35348. Thus, different IPSPs exert a strong control of the granule cell excitability on a timescale from ms to sec.


The level of depolarization at the soma of a Purkinje cell is strongly dependent on the inward and outward currents distributed over the large area of its dendritic tree. Here we analyze the relative contribution of synaptic and voltage-gated currents on the spiking behavior of the soma, using a previously constructed realistic Purkinje cell model (De Schutter and Bower, 1992). We find that dendritic α currents tend to depolarize the cell in a positive feedback loop, which is out of phase with an outward current system that is necessary to produce physiological somatic spiking rates (0.1-100 Hz). In the absence of inhibition, bursting behavior results. This modeling result is supported by intracellular recordings in vivo showing somatic bursting behavior when inhibition is blocked with bicuculline. The control of spike timing in the model was investigated by varying inhibitory and excitatory input rates and synaptic strength. The regularity of spiking was found to be strongly dependent on the temporal structure of both inhibitory and excitatory input.
111.13 SHORT-TERM INTERACTION BETWEEN THE COMPLEX SPIKE AND GRANULE CELL INPUTS IN A PURKINJE CELL MODEL

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We used a detailed compartmental model of a Purkinje cell (J. Neurophysiol. 71:379-401, 1994) to explore how a climbing fiber (CF) input modifies the response to subsequent granule cell (GC) inputs. We have shown that somatic responses to small synchronous GC inputs are amplified by dendritic calcium channels (PC in pressure), the same P channels are also activated by the complex spike (CS).

A CS fired to 100 ms before the synchronous GC input had no effect on the response of somatic EPSPs. A stimulus between 160 and 140 msec after the GC input was slightly potentiated. Between 140 msec and 90 msec there was a depression of up to 17%, but 80 to 60 msec after the GC input there was a progressive and large potentiation (90% at 60 msec) of the EPSP. GC inputs arriving sooner after the CS input fell into the prolonged afterhyperpolarization after the CS. Similar effects were seen for single spike (SS) firing in response to steady asynchronous parallel fiber inputs. Between 60 and 80 ms after the CS, SS frequency increased up to 50%, between 80 and 120 ms SS frequency decreased up to 35%.

These results may explain some of the conflicting results found with in vivo recordings. We conclude that, even without synaptic plasticity and without changes in GC firing patterns, a CS might cause either a potentiation or a depression of SS firing, depending on which time frame after the CS is considered.

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111.15 A NEW SILICON MICROELECTRODE ARRAY TECHNOLOGY FOR MULTICHANNEL EXTRACELLULAR RECORDING


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We have developed a new technology for the fabrication of micromachined in vivo extracellular microelectrode arrays. These devices ("probes") consist of thin films of metal deposited on a single-crystal silicon substrate which is thinner than 24 μm. Their overall shape is comblike, with each shaft of the comb supporting several individual electrode sites. In a neurophysiological experiment, a probe would be inserted into a differential animal, allowing simultaneous recordings to be made from multiple locations both normal and parallel to the brain surface. Our efforts complement work on similar devices performed in other laboratories.

We are drawing on our combined experience as neurophysiologists and engineers to optimize the utility of this technology for the end users. For example, we are concentrating on providing a large number of electrode sites on a shaft of microfabricated comb by using two metal layers, one for high-resolution interconnects and the other for electrode sites. This allows us to position the electrodes over the interconnects, placing 4 electrode sites along the cantilever of a shaft that is 24 μm wide, or 8 sites on a 40 μm shaft. Like other advanced probes, ours have indium electrode sites, which can be used for both recording and stimulating. We are also customizing the probe geometries to meet the needs of specific experimental paradigms. For the first fabrication run, we have designed 15 different probes, including some specifically for the rat cerebellar cortex, some for the rat olfactory bulb, and some for general-purpose use. Supported by a grant from Medtronic Corporation to J.B., and an NSF NYI award to G.K.

111.16 COMPLEX SPIKE SYNCHRONY OF PURKINJE CELLS IN THE VESTIBULOCEREBELLUM OF THE RABBIT

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By recording from Purkinje cell pairs in the ventral nodulus of ketamine- anesthetized rabbits, we showed that Purkinje cells in the same sagittal zone and having the same complex spike (CS) response characteristics, exhibit CS synchrony, that is the tendency to fire within 2 msec (Soc. Neurosci. Abs. 22:729). In the present study we show that CS synchrony occurs between cells in spatially separated zones but with the same class of CS responses, and between cells in the same zone but with different class of CS responses. Furthermore, we show that exhibit CS modulation in response to optokinetic stimulation (OKS) about the vertical axis are found in 4 separate areas which span most of the cerebellum, 2 in the nodulus and 2 in the flocculus (VA zones). We recorded in the nodulus from 16 Purkinje cell pairs consisting of one cell in each of the VA zones. Six of these pairs showed CS synchrony. In addition, 3 of 14 pairs consisting of a VA cell in the nodulus, and a VA cell in the flocculus showed CS synchrony. The HA zones of the nodulus and nodula contain contra-45° and ipsi-135° cells, both of which are modulated by OKS about a horizontal axis perpendicular to the ipsilateral anterior canal. They differ with respect to (1) ocular dominance, and (2) the origin of their CF input, which is the rostral dorsal cap and ventral posterior outgrowth, respectively. Of 7 contra-45°/ipsi-135° pairs, 5 showed CS synchrony. To summarize, CS synchrony is neither limited to Purkinje cells within a single sagittal zone nor to one class of Purkinje cells within the same zone.

111.17 ZONAL PROJECTION OF THE VENTRAL NODULUS IN THE RABBIT

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The projections of Purkinje cells from zones in the ventral nodular zone of the rabbit were studied with the use of extracellularly injected biocytin as an anterograde tracer. The zones were neurologically identified according to the complex spike (CS) modulation of Purkinje cells in response to optokinetic stimulation (OKS). Purkinje cells in the most medial zone (zone NR) do not respond to OKS, they project to the fastigial nucleus, peri-fastigial white matter, peri-interposed white matter (pwm), and medial vestibular nucleus (MVN). In the adjacent zone (zone VA) Purkinje cells respond best to OKS about the vertical axis, they project to the plvm and MVN. Purkinje cells in the next zone (zone HA) respond best to OKS about a horizontal axis perpendicular to the anterior canal, they project to the plvm, dorsal group y, superior vestibular nucleus (mainly peripherally but also centrally), and MVN. In the most lateral zone, (zone VA2), Purkinje cells project best to OKS about the vertical axis, they project to the plvm, dorsal group y, and MVN. Although all zones projected to the MVN, the projection from zone HA was largely to the caudal MVN, whereas the projections to the rostral MVN projected to the rostral and caudal MVN subdivisions. A few terminal fields were found in the descending vestibular nucleus, magnocellular MVN, interposed nucleus, and ventral dentate nucleus. No terminals were found in the prepositus hypoglossus or lateral cerebellar nucleus. The majority of axons innervated more than one nucleus. Often, three or four different areas received terminals from a single Purkinje cell axon. The zonal projection pattern of the ventral nodulus is compared to that of the flocculus, which, with respect to the visual climbing fiber afferents, has similar zones.

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CEREBELLUM: BEHAVIORAL NEUROPHYSIOLOGY AND CLINICAL STUDIES
FRIDAY AM

712.1

We determined the maximum activation direction (MAD) vectors of 54 neurons in the vestibulocerebellum in the alert cat. The cat rotated sinusoidally at 0.5 Hz in conditions of total darkness and/or dim light. We characterized neurons during vestibulocerebellar rotations about 3-12 axes. Of the 50 neurons recorded in dim light, 19 exhibited both simple and complex spikes and were classified as Purkinje cells (PC). As in our findings in decretebrate cats (SciNeuro 18/40), these PCs formed the internalized convergence in alert cats. A total of 40/50 neurons (17/19 PC) had MAD vectors >20° from any single canal plane. MAD vectors of the remaining 20 PC were >20° from ipsilateral horizontal canal (1 PC) >20° from ipsilateral anterior canal and 2 neurons >20° from contralateral posterior canal. This study suggests that PCs may be involved in the coding of gaze for smooth pursuit movements. Supported by EY07452, EY06452.

712.3
CONTEXT-DEPENDENT MODULATION OF CEREBELLAR NUCLEAR NEURONS RELATED TO THE PERFORMANCE OF SPECIFIC MOVEMENT SEQUENCES. M.S. Milik*, V. Bracha, J.B. Bloedel, Barrow Neurological Institute, Phoenix, Arizona 85013.

This study addresses the hypothesis that the modulation of some cerebellar nuclear neurons related to the performance of a particular component of a complex movement reflects the aspect of the overall movement being performed. Initially cats were trained to reach for a manipulandum and move it through a template consisting of a single straight segment. Trained animals were implanted with a multiple single unit, microelectrode-based, recording system targeted to the anterior and posterior regions of the fastigial and dentate nuclei and the anterior and posterior interposed nuclei. After recovery, the animal was required to learn a succession of new tasks, each consisting of 2 to 3 sequential, straight segments. The straight segment on which the animals were trained initially was a component of each new task and was always positioned in the same location in the silicentric world space coordinate. Histological estimates were drawn from the activity of simultaneously-recorded units held throughout the acquisition and performance of all the templates used over one experimental day. Analysis of specific movement or premovement-related unitary response components were relative to a movement segment common to all of the performed templates.

Of the 60 units recorded in seven experiments, 38% showed modulation changes dependent on the context in which the movement segment was performed. In some units these changes were related to movement components executed approximately 400 msec after the peak time of the unitary response. This finding suggests that these neurons participate in the specification and/or organization of movement sequences required for the performance of goal-directed limb movements. Supported by NHI grants NS30013 and NS21958.

712.5

Climbing fiber responses of Purkinje cells (PC) were recorded in the vermal, intermediate and lateral regions of a monkey trained to perform rapid flexions or extensions of the elbow in response to tone presented visual, auditory and somatosensory cues. 119 of the 314 recorded cells were time-lock activated with the movement onset in all triggering modalities. Two types of PC were defined: 1. Pre-onset CPR: CPR frequency increasing only before the movement onset (65/119 cells) with a mean latency of 39 ms (+/- 18 ms) and 2. Post-onset CPR: CPR frequency increasing at or after the onset of the movement (54/119 cells) with a mean latency of 111 ms (+/- 12 ms). In both cases, mean latency were statistically compared. In pre-onset CPR, the results showed a significant difference in the mean latency between the three areas investigated: Frontal and 2/3 - 41 ms (+/- 37 ms), Paramedian and Vermian lobes V-IV-V - 48 ms (+/- 24 ms), (P<0.0001). There was no difference in the mean latency of the post-onset CPR in any of the cerebellar regions investigated. Since the post-onset CPR are implicated in the control of the initiation of the movement because these cells are activated well before the onset of the movement. Post-onset CPR are probably more involved in feedback relative to the execution of the movement. Pre-onset CPR are activated earlier in the lateral cerebellum (Crus I and II) which project to the Destrata nucleus. Lateral cerebellum may be a part of a cortical trigger according to an hierarchical organization of the sequence of activation of the olivocerebellar fibers. Supported by MRC grant of Canada.

712.2

The aim of the present study was to determine the extent of the cerebellar interposed nucleus (IN) in the control of the classically conditioned and unconditioned reflexes elicited in three distinct effector systems. In food-motivated cats, instrumented conditioned to maintain quiescence on small platforms, three types of classically conditioned reflexes were established: 1) eyeblink reflex; 2) forelimb withdrawal reflex; and 3) hindlimb withdrawal reflex. Each animal was chronically implanted with a matrix of guiding cannulae and recording needles. The effects of temporarily inactivating IN subregions were systematically examined using microinjections of muscimol (1 μl, 80 ng). Transient inactivation of the IN affected conditioned as well as unconditioned components of the withdrawal responses in all three reflex systems. The drug injections altered both postural and phasic movement components of the limb's flexion responses. From the magnitude of the effects produced at different injection sites on the three conditioned reflexes, a topographical organization was derived. In addition, IN inactivation severely affected performance of the postural placement response in both limbs ipsi- lateral to the injection site and the tactile placating, hopping and magnus responses in the forelimb. The data support the view that various regions of the IN are differentially involved in the control of a variety of conditioned and unconditioned cutaneous-muscular and postural reflexes. Supported by NIH Grants NS30013, NS 21958, Max Kade Foundation.

712.4

Because the nucleus interpositus anterior (NIA) receives primarily cutaneous input from the rostral dorsal accessory olive while the nucleus interpositus posterior (NIP) receives primarily proprioceptive input from the medial accessory olive, we anticipated that the NIA and NIP might be useful for decoding movement. A macaque fuscataleus was trained in a pointing to target task, a reach to pull task, and retrieval of food from Kluver board, bottles or experimenters' hands. The approximate locations of the NIA and the NIP were mapped out during a number of penetrations where single units were recorded and related to the target task. Inactivations were induced by pressure or micromer inj. (2.0 ML), with the following times: Local anaesthesia for times four to five minutes, i.e. 1500 μg/ml and 2.5 minutes, i.e. 2 mg/ml). The data observed were described on the site of inactivation. The NIA inactivation greatly impaired the monkey's ability to plan and perform the movement during the pointing task or reaching the target board prior to grasping to pull the task. Once contact was established with the surface, the monkey appeared able to use cutaneous cues to improve hand function. The monkey was able to aim its arm and place the hand in the desired spatial location for use. NIP inactivation greatly affected the monkey's ability to aim its hand in the desired location in space or maintain the position during the use of hand. The monkey did exhibit proper hand shaping for the tasks at hand. The monkey appeared unable to use proximal forelimb-proprioceptive input and attempted compensation by using visual cues or tactile fixation. These observations suggest that the NIA contributes to the optimal function of the hand during tasks that require coordinated use of the fingers and hand and that the NIP contributes to the overall coordinated use of the hand by coordinating the transport of the hand to where it needs to be in space (aiming the hand) and maintaining its position once there so that the hand and fingers have a stable base to work on. Supported by N00014-90-J-1822 and 1 P50 NS44183-01.

712.6
NEURONAL CORRELATES OF DIRECTIONAL INFORMATION IN THE CEREBELLAR CORTEX DURING PREPARATORY AND DURING MOVEMENT. J.Gerin*, C.V. Kandler, C.R.S. N. Dep. de physiologie, Univ. de Montreal.

In order to assess the activity of cerebellar nuclear and cortical neurons during an instructed delay period two monkeys were trained according to the following paradigm. A 1000 ms or a 400 Hz tone were used as auditory instructions for right and left elbow flexions or extensions in response to tone. Tactile and auditory sensory cues were also recorded in response to tone presented visual, auditory and somatosensory cues. 119 of the 314 recorded cells were time-lock activated with the movement onset in all triggering modalities. Two types of CPR were defined: 1. Pre-onset CPR: CPR frequency increasing only before the movement onset (65/119 cells) with a mean latency of 39 ms (+/- 18 ms) and 2. Post-onset CPR: CPR frequency increasing at or after the onset of the movement (54/119 cells) with a mean latency of 111 ms (+/- 12 ms). In both cases, mean latency were statistically compared. In pre-onset CPR, the results showed a significant difference in the mean latency between the three areas investigated: Frontal and 2/3 - 41 ms (+/- 37 ms), Paramedian and Vermian lobes V-IV-VI - 48 ms (+/- 24 ms), (P<0.0001). There was no difference in the mean latency of the post-onset CPR in any of the cerebellar regions investigated. Since the post-onset CPR are implicated in the control of the initiation of the movement because these cells are activated well before the onset of the movement. Post-onset CPR are probably more involved in feedback relative to the execution of the movement. Pre-onset CPR are activated earlier in the lateral cerebellum (Crus I and II) which project to the Destrata nucleus. Lateral cerebellum may be a part of a cortical trigger according to an hierarchical organization of the sequence of activation of the olivocerebellar fibers. Supported by MRC grant of Canada.
111.7


We have used a direction-distance arm reaching paradigm to study the spatial characteristics of the responses of cerebellar Purkinje cells and quantitatively analyzed the simple spike response with univariate and multivariate regression models. The paradigm involved a multijoint movement in the horizontal plane from a centrally located start position to 48 targets located in different directions and 6 distances. Simple spikes firing in relation to direction, distance, and target location were analyzed using regression models as previously applied to the primate and primary motor cortex (Fu, et al., J. Neurophysiol., 70:2097-2118, 1993).

Based on a univariate model, the simple spike firing of the majority of cells (114/151, 75.5%) was modulated by movement direction, distance or both parameters. Of these cells, 31/114 (27.2%) showed a cosine-tuned modulation with direction and 45/114 (39.6%) cells were linearly correlated to distance, while 38/114 (33.3%) cells were modulated by both parameters. However, the majority of cells exhibited directional modulation at only one distance or distance modulation along one direction. Multivariate analysis indicated the firing of 99/128 (77.3%) cells was correlated with either direction, distance, or target location parameters (62/99, 62.7%) or some combination of these three parameters (37/99, 37.3%) The mean number of the multiple regression for the model was 0.34±0.15, considerably lower than the average of 0.61±0.18 obtained from the cerebral motor cortical area. Although significant correlation of simple spike discharge with kinematics occurs, still other parameters or models are needed to fully describe these cells' activity. Supported by NIH grant NS-18338.

111.8

SYSTEMATIC ERROR IN MULTIJOINT COORDINATION IN CEREBELLAR ATAXIA.

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In patients with ataxia, movements of daily living are performed with a characteristic pattern of slow and accurate movements. A systematic error in movements of daily living occurs with patients with ataxia, but the type of movement which is altered by ataxia is not well documented. The purpose of this study was to analyze the pattern of movement of daily living in patients with ataxia. The study was performed with a two-dimensional computerized system (E.L.I.T.E) and movement parameters were collected from the trunk and the upper extremities.

The study was performed with a two-dimensional computerized system (E.L.I.T.E) and movement parameters were collected from the trunk and the upper extremities. The results showed that patients with ataxia had a higher systematic error in the movement of the upper extremities than in the trunk. This is consistent with the clinical observation of patients with ataxia who have a higher systematic error in the movement of the upper extremities than in the trunk. The results of this study suggest that the systematic error in movement of daily living may be a result of a deficit in the control of movement.
T11.3
NADPH-DIAPHASE ACTIVITY IN DEVELOPING CEREBELLUM OF THE CHICK
C.R. Durkin*, A. Shastak* (SPONC European Neuroscience Association) Dept. of Biology, Crete Univ., Iraklion 711 06, Crete, Greece.
A novel messenger, nitric oxide (NO), has been suggested to play a role in synaptic plasticity and to participate in shaping neuronal connections. In order to investigate the role of NO in the developing cerebellum, we studied the distribution of nitric oxide synthase in this region. From embryonic day 9 (E9) until E15, the most prominent feature was the strong NADPH-D activity in the principal neurons of the cerebellar white matter, which contained a NADPH-D-positive ascending axons, that targeted selectively the developing folia and that Orli-like stained neurones moving onwards from neocortical to cerebellar cortex. In addition, the NADPH-D-positive networks were formed in the brainstem cerebellar nuclei and in the thalamus. The development of NADPH-D activity was more advanced in E17. This suggests that the NADPH-D activity appeared in the cerebellum as a result of the intrinsic development of the cerebellar axon.
T13.7

Cerebellar nuclocortical projections have been reported to be topographically ordered to both the olivocerebellar nuclei and the somatotopic arrangement of the olivocerebellar input. This study was aimed to address two related questions, i.e. whether or not the topographically homologous areas of the anterior lobe (PIAL) and paramedian lobule (PML) - in C1, C2 and C3 compartments - differ in the extent and topology of their nuclocortical input; and II) whether or not there are barbell neurons that connect by way of branched collaterals these two areas, as it was previously found for the olivocerebellar system. With this aim, one of the fluorescent retrograde tracers Fast blue (FB) and DiAmdino yellow (DY) was injected into the forelimb-related folia (FL) of the intermediate compartments of either PIAL or PML.

The analysis of single- and double-labeling in the inferior olive complex (IO) allowed to verify that the FB and DY injected areas were centered in the FL areas, and only those experiments in which the two IO labeled populations were in register, were selected for the present study. The results show that the FL-PIAL and FL-PML areas in C1, C3 and C2 cortical zones are projected upon by neurons located in the anterior (NIA) and posterior (NIF) interposed nuclei, thus roughly reciprocally the cortico-cerebellar organization. Within these nuclei, the two populations of labeled neurons overlap and show a topographic relation to the cortical somatotopy. In the areas of overlap, double-labeled neurons are found. A minor non reciprocal projection stemming from the fastigial nucleus, and a contralateral projection from the interposed nuclei was also observed.

These data are taken to indicate that the corticoepitodal fibers from the interposed nuclei to C1, C2, C3 compartments mainly operate as a closed loop feed-back system. The nuclocortical projections may be seen as a system which allows communication among different compartments.

T13.8
VISUAL INPUT TO THE CEREBELLUM OF RATS. B. Mercier, E. Jenkinson, I. Krall-Hans, C. Swales and M. Glickstein* Dept. of Anatomy, University College London, Gower Street, London WC1E 6BT.

To trace visual pathways to the cerebellum we injected wheatgerm agglutinin (WGA-HRP) into the visual cortex or the superior colliculus of rats and plotted retrograde distribution of fibers in the pontine nuclei and nucleus recessus segni pontis (NRP). In parallel experiments we injected WGA-HRP into the cerebellar lobules VII or IX of the vermis or the petrosal lobule, and plotted retrogradely filled cells in the Pons. The visual cortex projects principally to a dorsalolateral region in the rostral half of the pontine nucleus. Although collicular projection overlap somewhat with those from the visual cortex, they extend far more caudally. Unlike the visual cortex, the superior colliculus also projects to NRP. We compared the location of retrogradely labelled cells following cerebellar injection with the results of studies of orthograde projections from cortex and colliculus. The petrosal lobule receives its input from the same region of the pontine nuclei which receives fibers from the primary visual cortex. The region of pontine nuclei whose cells project to lobule IX receives inputs from both visual cortex and superior colliculus. Unlike the petrosal, lobule IX, lobule VII receives a major input from NRP. The results suggest that there are two relatively independent pathways by which visual information can reach the cerebellar cortex. One originates in the primary visual cortex and projects by way of the rostrolateral part to the petrosal lobule and lobule IX. Another originates in the superior colliculus and projects by way of NRP to lobule VII. The pattern of organization is similar to that seen in parallel experiments with monkeys.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 20, 1994
T14.1 CORTICOTROPIN-RELEASING FACTOR (CRF) AXONS AND BINDING SITES IN THE KEELER CEREBELLM. J. S. King*, G. A. Bishop, and P.C. Madden, Jr.* Dept. Cell Biology, Neurobiology and Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

It is our working hypothesis that CRF functions in early development as a growth regulating factor. We previously reported in the developing osprey cerebellum that (1) CRF is present in early-born (G8 and growth cones) and (2) the initial CRF binding sites are expressed over Purkinje cells subsequent to their migration (1-3). In this study we used an antibody to CRF and 125I labeled CRF to describe the distribution of CRF labeled axons and CRF binding sites in the cerebellum of the domestic mouse, reeler. In the reeler cerebellum, most Purkinje cells fail to migrate to the typical monolayer and are misplaced in the internal granule layer or in three paired deep cellular masses. CRF immunoreactive axons terminate in the 3D arrays as climbing fibers and mossy fibers, but only in the reduced molecular and infragranular layers. In contrast, CRF binding sites are present over Purkinje cells that fail to reach the minus and in the deep cellular masses. Taken together, these findings are compatible with a role for CRF as a neuromodulator. CRF receptor binding is expressed over Purkinje cells only after they reach the cortical surface, and supports our working hypothesis. (Supported by NS-08798).
CEREBELLUM: PHARMACOLOGY


The cerebellar mossy fibre system is made up of very fine fibres from the medial vestibular and prepositus hypoglossi nuclei, and thin varicose fibres of which the origin is still uncertain. Furthermore, cholinergic agents administered muscarinic receptor antagonists reveal aversive, modification of the physiological studies, however, have yielded conflicting results about the identity of the cholinocceptive elements. To characterise cerebellar cholinocceptive elements the ultrastructural level, antibodies against cholinergic receptors protein have been used. Muscarinic receptor protein immunoreactivity has been found in astrocytes. In the granular layer some Golgi cells, mossy fibre terminals, and granule cell bodies also express this staining. Some Purkinje cell somata display cyttoplasmic labelling but most of these perikarya receive labelled axonal terminals. In the molecular layer prominent mossy receptor immunolabelling has been observed in some basket cells, Bergman glia parallel fibres. Nicotinic receptor protein is differently expressed. Some granule and Golgi cells, but no astrocytes or mossy fibre terminals, express immunoreactivity. Most Purkinje cells and their dendrites show strong staining. In the molecular layer parallel fibre varicosities, but no Bergmann glia fibres, express nicotinic receptor protein. Species differences exist in topography of both muscarinic and nicotinic stained cerebellar profiles. The results indicate that muscarinic and nicotinic receptors are synthesised by multiple and distinct cerebellar cells. Combined with the species differences this might explain the present inconsistent data on the identity of cerebellar cholinocceptive profiles.


The unipolar brush cell (UBC), a previously neglected small neuron of the cerebellar granular layer, is densely concentrated in the vestibulo-cerebellum and is characterized by its single brush-like dendritic arbor arising from a short stalk (Mugnaini and Pizzcoro, J. Comp. Neurol., 339: 174-180, 1994). The UBC is innervated by one or two prominent axonal collaterals near the soma. Morphological and immunochemical studies have shown that the UBC is uniquely associated with the glutamatergic (AMPA) network of the cerebellum. By using an antibody specific for AMPA, kainate and NMDA receptor subunits we immunocytochemically identify the receptor's association with the UBC synapse. Frozen and Vibratome sections of rat and cat cerebellum, perfused with buffered aldehydes, were incubated with polyclonal antisera to GluR1, GluR2, GluR4, GluR6/7, KA-2, NMDA-R1 and NMDA-R2A (kindly donated by R. Whittall), diluted 1:50 to 1:200, and with monoclonal antibodies (mab) recognizing GluR5/6 (Clone 4F5, Pharmingen) and NMDA-R1 (clone 54.1) both diluted 1:50 to 1:2000. The PAP or the APC method were employed using DAB as the chromogen. UBCs were strongly immunostained with the UBCs. At the electron-microscopic level, GluR2/3 and GluR5/6 immunoreaction products were found throughout the UCB cytoplasm. The mAb to GluR5/6 stained the post-synaptic densities at the mUCB contact more densely than the polyclonal GluR2/3 antisera. Thus we were able to clearly identify both AMPA and kainate glutamate receptor subunits at the m-UCB synapse. Supported by USA PHS grant NS 09094.

T14.9 SPATIOTEMPORAL STUDY OF F0S ACTIVITY IN THE INFERIOR OLIVE AND CEREBELLUM FOLLOWING NOS EFFECT OF SLOWING CPU 1. D. W. Sexton* and A. J. Beitz, Department of Vet.Pathology, University of Minnesota, St Paul, MN 55108.

The tenegeome alkaloid, harmaline, when given systemically produces repetitive synchronous activity in neurons of the inferior olive (IO) and presumably release of excitatory amino acid neurotransmitter at the olivo-Purkinje cell synapse. Following subcutaneous injections (25 mg/kg in saline) single unit activity was recorded in the IO before and during 15 min to 24 hours, rats were perfused with paraformaldehyde, sectioned and reacted for F0S protein immunohistochemistry. The results indicate that as early as 15 min and 24 hours following harmaline there are detectable levels of FOS in caudal subdivisions of IO (i.e., IOA, B, C) and that by 30 min most other subdivisions of IO (i.e., IOO, IOM, IOP, IOR) were also stained. Activity peaked by 7 hours with regard to numbers and intensity of positive FOS nuclei and had returned to control levels by 18 hours post-harmaline. At 24 hours subdivisions of IO were found to contain F0S activity for prolonged times after lateral IO subdivisions returned to control levels. The cerebellar cortex showed marked increase in F0S activity by 1 hour post-harmaline. Throughout the cerebellum and the Purkinje cell (PC) layer of the vermis and paravermis showed F0S mRNA staining. A subset of PC in the vermis and paravermis were found to display F0S activity at 1 hour and all layers in the molecular layer (ML) were numerous in the vermis but sparse in the lateral hemispheres. Labeling in the ML peaked by 2 hours and gradually returned to control levels by 12 hours post-harmaline. The deep cerebellar nuclei also showed spatial and temporal changes in F0S activity. A morphologically diverse group of neuronal nuclei were evident in the fastigial nuclei by 30 min, but changes were in no particular group of small nuclei by 18 hours. The interposed and lateral cerebellar nuclei displayed intense F0S activity by 2 hours and returned to control levels by 24 hours post-harmaline. Taken together these results suggest that there are distinct spatial and temporal changes in the cortex, IO and deep cerebellar nuclei in response to harmaline activation of the IO. Supported by Grant # NS31188 and DC01086.


The major cerebellar afferent pathways, climbing and mossy fibers, are reported to use an excitatory amino acid as their neurotransmitter (chemical lesions of the chemical lesions of the pontocerebellar mossy fiber tract were made in an effort to elucidate the receptor subtypes responsible for mediating cerebellar afferent neurotransmission. Intraplural (IP) injections of 50 to 60 mg/kg 3-acetylpyridine (3-AP) were used to chemically lesion the inferior olivary nuclei (500; 250; 125 mg), male SD rats. The right middle cerebellar peduncle (MCP) was lesioned with a horizontal sweep of a 3-AP needle inserted through the occipital skull plate. In situ hybridization histochemistry was used to monitor post-synaptic changes in the levels of mRNA for subunits of the NMDA (NR1), AMPA (GluR1) and metabotropic (mGluR1) subtypes of glutamate receptors. In addition, immunocytochemistry with specific antisera for the NMDA and AMPA receptors (GluR2/3) and GluR4 was used to detect differences between control, 3-AP-lesioned and MCP-lesioned rats. Preliminary results suggest that GluR2/3 immunostaining in subpopulations of Purkinje cells oriented along parasagittal zones becomes more intense in the 3-AP-lesioned animals. In non-lesioned controls, all Purkinje cells appear to be uniformly stained. These results suggest a selective glutamate receptor subtype upregulation after climbing fiber destruction. Supported by NS31818.

T14.12 EVOKED RESPONSE MODULATION IN INTRACLINELLARLY RECORDED CEREBELLAR PURKINJE CELLS BY SEROTONIN. L. J. Larson-Pror* and R. M. Buskey, Dept. of Neuroscience and Anatomy, Penn State College of Medicine, M.S. Hershey Medical Center, Hershey PA, 17033.

Distinct differences in the extent and distribution of serotonergic input to the cerebellar cortex have been reported for several mammalian species based upon the extent and distribution of 5-HT1A binding sites. The physiologic responses of extracellularly recorded cerebellar Purkinje cells in exogenously applied serotonin have been reported to be variable, eliciting both increased and decreased spontaneous firing rates. Recently, the 5HT1A receptor has been implicated in the reduction of Purkinje cell activity in young (150 to 250 g), male SD rats. The right middle cerebellar peduncle (MCP) was lesioned with a horizontal sweep of a 3-AP needle inserted through the occipital skull plate. In situ hybridization histochemistry was used to monitor post-synaptic changes in the levels of mRNA for subunits of the NMDA (NR1), AMPA (GluR2/3) and GluR4 was used to detect differences between control, 3-AP-lesioned and MCP-lesioned rats. Preliminary results suggest that GluR2/3 immunostaining in subpopulations of Purkinje cells oriented along parasagittal zones becomes more intense in the 3-AP-lesioned animals. In non-lesioned controls, all Purkinje cells appear to be uniformly stained. These results suggest a selective glutamate receptor subtype upregulation after climbing fiber destruction. Supported by NS30759.

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715.1 IDENTIFICATION OF PASSIVE AND REFLEX COMPONENTS OF HUMAN DYNAMIC ANKLE STIFFNESS. R.E. Kearney, R.B. Stein* and L. Parengwaian.

The role of stretch reflexes in the control of movement and posture remains unclear since it is difficult to separate the relative contributions of passive and reflex mechanisms to the torques generated in response to a joint perturbation. System identification techniques, using random perturbations, have yielded good descriptions of overall joint stiffness and reflex EMG dynamics but have had little success in characterizing the reflex contribution to the stiffness. Recent evidence suggests that the reflex response is strongly nonlinear and that on-going movements inhibit reflex action in proportion to their average velocities.

This manuscript summarizes our approach to studying ankle stiffness using experimental and analytic methods designed to overcome these problems. We have used position perturbations which were designed to provide a wide-bandwidth position input with low average velocity. Possible nonlinearities in stiffness dynamics were accounted for through the use of a parallel-cascade system technique for non-linear system identification. Experiments using these new methods consistently identify two distinct components in the overall stiffness dynamics. The first component is position-dependent: acts at short-latency (< 40 ms) and displays high-pass dynamics; it seems certain to reflect the passive contribution to stiffness. The second component is velocity-dependent, acts at longer-latency (> 400 ms) and displays a dynamic non-linearity followed by low-pass dynamics; this component likely represents the reflex contribution to ankle stiffness. The reflex contribution to stiffness was most significant at low size relative to the passive contribution, and varied strongly with the parameters of the perturbation as well as joint position and level of activation. Thus, the role of stretch reflexes in motor control is likely to be very context sensitive. (Supported by the Medical Research Council of Canada)

715.3 MOTOR UNIT ACTIVITY DURING SPASTICITY. R.H. Ross and C.K. Thomas.* The Miami Project to Cure Paralysis, University of Miami School of Medicine, Miami, FL 33136.

Spasticity is a common phenomenon following human spinal cord injury. Most studies to date have examined changes in reflex pathways in individuals who experience spasticity but have been concerned with the use of anti-spastic agents. Thus data are therefore provide important clues about the baseline condition from which spasticity stems. However, they provide little information about the processes which occur during spasticity per se. The study described here was undertaken to examine the patterns of motor unit activity during induced spasm. Seven individuals with chronic (> 1 year post injury) spinal cord injury between C5-C6 induced spasm by gentle touch to the face/cheek of the upper body. The electromyogram (EMG) was recorded unilaterally from the surface of each of the triceps surae muscles and intramuscularly from the medial gastrocnemius muscle. None of the triceps surae muscles were used for motor control. However, during spasm, there were usually bursts of EMG in all three muscles. Occasionally, periodicity in rhythmic clonic activity or motor unit activity were observed. During the brief and sustained spasm, motor unit patterns were evident: (1) at slow tonic rates (<1 Hz), 2) with repetitive discharges, and 3) with an increase then decrease in rate as the spasm reached its peak. The first two patterns of unit activity seem unique to involuntarily contractions whereas the third pattern is typical of motor unit behavior during voluntary contractions. During clonus, motor unit activity also rose and fell rhythmically with the repetitive rise increase in surface EMG. Peak rates for different motor units occurred in these phases. These data that the asynchronous versus synchronous activity of different motor units may explain the natural balance of the spasm (brief burst versus clonic form).

715.5 Interaction of Flexion (FR) and Crossed Extension Reflexes (CER) with Tilt-Evoked Responses (TER) in Standing Humans. P. Nagel* and C.W. Hui-Chan.* School of Phys. and Occ. Therapy, University of Western Ontario, London, Canada; N6H 5C1.

With the use of isometric, ankle and foot somatosensory inputs were found to be contributory to the early component of TER in lower limb muscles (Bussel et al., Prog. Clin. Neurophysiol., 835:325, 1989). The purpose of this study was to examine whether FR or CER inputs would modify the lower limb TER in a functionally meaningful manner. Thirteen young healthy subjects were instructed to stand on a tilting apparatus with straps and a head collar. They underwent sudden forward-body tilt of 15° with an axis of rotation co-linear with the ankle joint. The mean head acceleration was 0.7g, as measured with a linear accelerometer mounted on a dental lab and CERs were elicited with electrical stimulation (ES) of the right tibial nerve below the hairline (maximal stimulus intensity in the standing ES, 2/30, max trials). When the body was tilted forward, the measured acceleration of the ipsilateral tibialis anterior (TA) muscle was maintained at 70-80% of maximum voluntary contraction. The ES was delivered at the onset of the peak of the TA activation. During 30 ms of a 1 s ramp at 20 ms/s, the TA, soleus (SO), biceps femoris (BF) and vastus lateralis (VL) muscles were bilaterally recorded.

The FR area (n=3) did not modify the TER in the majority (n=57) of subjects whose TA FR area was significantly modified by tilt, while it was decreased in only 1 subject. The remaining 6 subjects showed no effect of tilt on the ipsilateral TA area (n=3), or less than 20% of FR occurring (n=3). Moreover, more subjects showed a response in the contralateral TA during tMS (n=4) than during ES (n=6) or tilt alone (n=11). Finally, a majority of responders had a tendency for a larger EMG area in the contralateral VL (n=9/10 responders), and contralateral SO and/or VL (n=5/6) during tMS than ES alone.

These results indicate that while head-and-body tilt tended to increase the excitability of lower limb flexor (including FR) and extensor (including CER) muscles activated by the flexion reflex system. The resultant co-contraction of ankle muscles and increased knee extension activation is consistent with the more difficult task of maintaining a stance against perturbation. This project was financed by Parkinson's Foundation of Canada and a MRC fellowship for N.P.
11.7.5 THE EFFECTS OF LOW INTENSITY CUTANEOUS STIMULATION ON THE H REFLEX MODULATION DURING STATIC AND DYNAMIC CYCLING MOVEMENTS. C. Labreque and M. Milette* Dép. de kinanthropologie, Univ. du Québec à Montréal, Montréal, Québec, Canada, H3C 3P8.

Numerous studies have shown modulations of the cutaneous reflex during rhythmic movements such as walking and cycling. Similarly, the H reflex has also been shown to be modulated by muscle activity. However, in real life situations, the nervous systems must consider these two sources of interacting feedback during the ongoing movement. The main objective of this study was to examine the effects of cutaneous stimulation on muscular inputs during stationary positions and active cycling movement. The cutaneous stimulation consisted of 10 ms male 1-1 ms pulses, at 2 times the sensory threshold, and applied to the dorsal surface of the foot. The H reflex stimulation consisted of a 1 ms pulse applied to the tibial nerve and the sciatic nerve. During the static condition, the soleus H reflex varied in amplitude depending on the phase, the largest one being at 90° and the lowest at 0°. The low intensity cutaneous stimulation had a tendency to increase the H reflex amplitude particularly at the 270° position. During the active cycling, the H reflex was also modulated, the greatest response being at 90° while the lower amplitude was observed at 0° and 180°. The H reflex continued to be modulated in a similar fashion following the cutaneous stimulation, however, the responses tended to be slightly inhibited. (Supported by FAPAC, UQAM)

11.7.6 SPASTICITY IN THE CONSCIOUS RAT: QUANTITATIVE EXAMINATION OF STRETCH AND VELOCITY SENSITIVE REFLEX COMPONENTS. M. Köhnen & J. A. F. Cole* Dept. Neuroscience, Univ. of Cambridge.

Although the rat has often been used as a model of spinal cord injury (SCI), no study has yet identified spasticity, defined as enhanced stretch activity and velocity-sensitive (VS) reflexes. We present preliminary data of a SCI model using the conditioned cat, which allowed us to study the relationship between stretch reflex activity following subcortical lesions in the upper lumbar level. Background forces were closely matched before and after SCI to obtain more direct measurements of test reflex activity. Injury-induced stretch reflex deficits were similar to those obtained previously in the conscious cat, with increases in both dynamic and static torque amplitudes at both the VS and dynamic index components. However, in contrast with the cat, hyper-reflexia was not observed in the first week post lesion, but developed to a maximum over the first month. We conclude that with appropriate reflex testing conditions rate can also be defined as spastic following selective spinal lesions, but that increased VS of long-latency stretch reflexes as a hallmark of SCI should be re-examined using other testing procedures. Supported by Grant NS27511 and NSF0.


11.7.8 IDENTIFYING THE INTERNEURONS INVOLVED IN THE REFLEX RESPONSE TO ELECTRICAL STIMULATION OF THE INTERNAL BRANCH OF THE SUPERIOR LARYNGEAL NERVE. Y. Takaoka, C.L. Ludlow and W.S. Sacktor, VSS, NIDCD, NIH, Bethesda, MD 20892.

Electrical stimulation of the internal branch of the superior laryngeal nerve (SLN) produces a bilateral adductor response in the larynx. The purpose of this study was to identify interneurons in the medulla oblongata that comprise this reflex pathway using the retrograde tracer cholera toxin B to label the nucleous ambiguus (NA) and the stimulation induced expression of the proto-oncogene, c-fos. Cholera toxin B was injected into the hypoglossal, lateral orbital, cuneate, and the spinal cord medulla oblongata (to identify the efferent neurons in the NA). After 72 hours, the cats were anesthetized with alpha chloralose and the right SLN was placed in a bipolar current electrode. Following 4 hours of stimulation, the cats was sacrificed and units were stimulated at 0.5 Hz at supra maximal levels for 20 minutes immediately before euthanasia. Serial 50 pm transverse frozen sections were made through the medulla oblongata. Odd numbered sections were processed for immunohistochemistry by means of anti-c-fos protein. Even numbered sections were incubated with anti c-fos-like antibody. As expected, cholera toxin B labeled neurons and dendrites in the NA and terminals in the NTS. C-fos like immunoreactive neurons were identified bilaterally in the NTS from the level of the most rostral portion of the dorsal motor nucleus of the vagus to the most caudal portion of the interolivary nucleus (IO), and in the NA from the rostral end of the hypoglossal nucleus to the caudal end of the IO. Bilateral c-fos like reactions were also observed in the reticular formation around the NA. Neurons expressing c-fos that lay outside the anatomically defined NA and NTS are candidates as interneurons.


Noise prepulses (PP) presented in a masking noise (N) just prior to a startle stimulus (SS) alters the facilitatory (PP) on startle (SS) acoustic startle (AS). There are many studies of the psychophysical and pharmacological characteristics of PP, but little is known about PP. Here we report that a 20 msec PP ending with the startle pulse facilitates the startle in inverse linear proportion to its signal to noise ratio (PN). Accordingly, our weakest PPF (+3 dB ratio) approximately doubled the AS to a 115 dB stimulus, whereas ratios greater than +9 dB resulted in no changes. Such responses may be due to a consistent PN ratio (a +3 dB difference) increased with increased levels from 30 to 40 dB, but then remained constant for higher intensities (tested to 73/70 dB). As PP levels increased, PP was enhanced to PPI beyond 60 - 80 ms. Both effects increased with experience. Dose-sensetive enhanced PPF (revealing that PPF is different from other forms of AS) but reduced PPI, EPP was increased by the PPI lead times as short as 35 ms indicates that these mechanisms have a peripheral nature. (Supported by FHS grants, AG09524 and MH43081)

11.7.10 SYMPAIES IN ROSTROLATERAL MIDBRAIN MEDIATE "FEAR" POTENTIATION OF ACOUSTIC STARTLE AND ELECTRICALLY evoked startle. J.S. Yeomans* and P.W. Frankland. Dept. Psychology, University of Toronto, Toronto ON, M5S 1A1.

Electrical stimulation of the caudal ventral amygdalofugal (VAF) pathway from the amygdala to the brain stem evokes startle-like responses, which are enhanced by acetylcholine. The lesion of this pathway eliminates the VAF to the brain stem response. Although the medial habenula and the ventral tegmentum have been proposed to mediate the startle response, the physiological basis of this effect has not been clearly determined. We have studied the effects of NMDA receptor agonists and antagonists on the startle response. The results indicate that the startle response is mediated by the ventral tegmentum and the habenula. The startle response was assessed in the rotarod and the wire suspension test. The results indicate that the startle response is mediated by the ventral tegmentum and the habenula.

The acoustic startle response (ASR) can be enhanced by conditioned or unconditioned fear. It has been shown that the central nucleus of the amygdala (CA) and a second pathway to the caudal pontine reticular nucleus (PnC), an essential part of the primary startle circuit, is important for the enhancement of the ASR. It was unclear, however, whether these modulations were directly mediated by the amygdalorhinal pathway or whether there exists a relay nucleus within this pathway. We tested the hypothesis that the midbrain central gray (CG) is important for the effects of fear on the ASR. Neuroanatomical tracing studies described a descending projection from the CA to that part of the CG where a descending projection to the PnC takes its origin. We lesioned this part of the CG with the neurotoxin quinolinic acid and then measured the effects of conditioned and unconditioned fear on the ASR. Lesions of the dorsal and lateral parts of the CG totally blocked the sensitization of the ASR by footshocks (unconditioned fear) and blocked the enhancement of the ASR by conditioned fear without affecting the ASR amplitude in the absence of the conditioned or unconditioned stimuli. This finding suggests a crucial role of the CG for the enhancement of the ASR by conditioned and unconditioned fear. The pathway from the CA via the CG to the PnC represents one part of a complex circuitry mediating the effects of conditioned and unconditioned fear to the ASR.

Supported by DFG (SFB 307) and GKN Tübingen.


The present findings represent part of a larger study investigating the contribution of pelvic innervation to reproductive processes in the rabbit. In adult chinchilla breed females, reflex EMGs of the Musculi constrictores vaalei and vestibuli, ischiocavernosus, bulbospongiosus, obturatorius internus, pubococcygeus, and rectus abdominis, were recorded in response to stimulation of the clitoral sheath, vagina, cervix, perineal, and perianal skin, and the flanks. Strong EMG activity was elicited by clitoral stimulation in all muscles except the rectus abdominis, and to a slightly lesser degree by stimulating the first and second thirds of the vagina using a glass rod. Both kinds of stimulation resulted in prolonged afterdischarges - up to 50 sec for the pubococcygeus and the obturatorius musculi. Stimulating the cervix produced a response only in the rectus abdominis with an afterdischarge of about 18 sec. Brushing or pressing the clitoral sheath, perineal or perianal skin resulted in EMG activity only in the pubococcygeus and, possibly, the constrictor vestibuli. Stimulating the flanks failed to elicit any response.

In summary: Clitoral or vaginal stimulation provoked strong EMG activity in almost all the muscles studied which was blocked by cervical stimulation. Withdrawal of cervical stimulation resulted in the reappearance of EMG activity which, however, was somewhat attenuated if clitoral stimulation was avoided.

SPINAL CORD AND BRAINSTEM: PATTERN GENERATION

BILATERAL MOTOR RHYTHMS DURING FICTIVE ROSTRAL SCRATCHING IN THE TURTLE. Paul S.C. Strick*, John C. Yescia, Eddie C. Field, and Scott R. Carney, 1Dept. Biology and Movement Science Program, Washington University, St. Louis MO 63130. 2Dept. Neuroscience, Univ. of California, Riverside, CA 92521.

Fictive rostral scratching is produced in a prone, immobilized turtle by gentle mechanical stimulation of the ipsilateral (ipsi) midbody sheath bridge (J. Neurophysiol. 53: 1517, 1985). Most previous work has focused on ipsi motor output. This output includes bursts of hip flexor (VP-HP) motor activity that rhythmically alternate with bursts of hip extensor (HR-KF) motor activity. Knee extensor (PT-KE) motor activity occurs during the latter portion of each burst of hip flexor activity. Martinez-Gomez and Strick (J. Exp. Biol. 164: 14 to 26, 1991) demonstrated rhythmic bursts of contralateral (contra) hip flexor activity which alternate with bursts of ipsi hip flexor activity during fictive rostral scratching. We add to these previous findings. Stimulation of the ipsi sheath bridge also produces a fictive motor pattern of contra hip extensor bursts that alternate with contra hip flexor bursts. Contra hip extensor bursts also occur during the early portion of each burst of hip flexor activity. Contra hip flexor activity begins during the later part of the ipsi hip flexor activity and continues during peri hip extensor activity. Simultaneous-bilateral stimulation of mirror-image locations, one in the left rostral scratch receptor field and the other in the right rostral scratch receptive field, can alter the right-left extent of rostral scratching motor patterns. The onset of bilateral scratching is a function of the degree to which the neurons in the pattern generator are activated. In summary, there is in-phase activation of left and right hip flexor nerves. After the first cycle of response and for the remainder of the response, the activity of each left motor pool alternates with the activity of its mirror image motor pool on the right.

The patterns of activity seen during bilaterally activated fictive rostral scratching are similar to the patterns observed during bilateral activity in rostral scratching in spinal turtles as well as during actual forward scratching in intact turtles (Field and Strick, this volume). These behavioral and spinal patterns may be mediated by an excellent spinal model system to examine spinal mechanisms for the coordination of left and right rhythmic motor behavior. Supported by NIH Grant NS30768 to PSGS.
716.3 DEPRESSION OF MONOSYNAPTIC GROUP II AND OTHER FIELD POTENTIALS DURING MLR-EVOKED FICTIVE LOCOMOTION SUGGESTS A REDUCTION OF TRANSMISSION IN SENSORY AFFERENT IN THE WALKING CAGE. S. J. Shefshak and D. McCreery. Dept. Physiol., Univ. of Manitoba, Winnipeg, Canada, R3E 0W3, and CIEA-IPN, Mexico.

The possibility that transmission from sensory afferents might be affected during MLR-evoked fictive locomotion was examined in decerebrate cats. Monosynaptic field potentials were evoked by stimulation of extensor (Q, G5), flexor (Sart, PBST) and cutaneous nerves and recorded in lumbar and sacral spinal segments. During MLR-evoked fictive locomotion monosynaptic components of group II field potentials were reduced up to 70%. In those trials where locomotion appeared several seconds after the onset of MLR stimulation, group II field potentials were maximally depressed only when the locomotor program was fully developed. Group I and cutaneous field potentials were also depressed but to a lesser degree. All monosynaptic field potentials were tonically depressed with the onset of fictive locomotion and for several seconds after its cessation. A smaller phasic depression during extension was often superimposed on the tonic depression. We suggest that both descending pathways and spinal locomotor networks contribute to the depression of transmission from group II, group I and cutaneous afferents during MLR-evoked fictive locomotion. Although the mechanisms have not yet been determined, presynaptic inhibition of the terminals of segmental afferents is likely. Supported by the MRC and the Rick Hansen Legacy Fund.


After step training, the hindlimbs of the spinal cat transected at T12-T13 can regain the ability for weight bearing locomotion on a treadmill. When an obstacle is placed in front of the foot during the swing phase of a step, the limb can be lifted above the obstacle after contact but before lift-off. The swing phase of a step can, however, be decreased by a light object placed in front of the foot. This object was introduced during the swing phase to perturb the hindlimb step cycle. The swing phase of the first step following contact of the hindlimb foot to the object was shortened to pre-contact steps. With repetitive obstruction, the step height increased in succeeding steps thus minimizing contact with the obstacle. In some instances, contact with the object led to complete avoidance in the locomotor-related cat spinal neurons. They show that a subpopulation of c-fos-immunoreactive cells are first-order interneurons and some may utilize acetylcholine, serotonin and GABA as neurotransmitters. Supported by MRC Canada, Human Frontier Science Program and the Health Sciences Centre Foundation.


The N-type calcium channel regulates the release of certain neurotransmitters, particularly acetylcholine, and is sensitively blocked by the Ca-channel blocker GVIA. We used this tool to investigate the fundamental role of the medullary Pre-Botzinger complex in the generation of respiratory rhythmic activity in adult mammals. Lesion experiments have demonstrated that the Pre-Botzinger Complex is critical for rhythm generation in neonatal rats. In adult animals it has been demonstrated that all types of respiratory neurons are present within this medullary area but their importance for rhythm generation remains uncertain. In this study we pressure injected u-conotoxin GVIA (a blocker of the Pre-Botzinger Complex) into the medulla of adult cats. Male and female cats were anaesthetized (Pentobarbitol 40mg/kg i.p.), paralyzed, vagotomized and artificially ventilated. Respiratory activity was monitored from four- or six-channel physiological nerves with or without intracellular recording of respiratory neurons. When injected into the Pre-Botzinger Complex, u-conotoxin GVIA had profound effects on respiratory activity. In this limited area blockade of the N-type calcium channel decreased the amplitude and the slope of phrenic nerve activity and increased significantly the cycle length. Prolonged injection induced central apnea. Although u-conotoxin GVIA was injected into the Pre-Botzinger Complex of one side, the induced respiratory effects were bilateral. All effects lead to a decrease of sympathetically induced drive potentials to late-inspiratory, post-inspiratory and expiratory neurons. We conclude that u-conotoxin GVIA blocks tonic excitatory drive to a specific area in the respiratory network of the Pre-Botzinger Complex. The data also demonstrate that in adult cats the Pre-Botzinger Complex is essential for respiratory rhythm generation.

716.6 ANTIDROMIC ACTIVITY OF DORSAL ROOT FILAMENTS DURING TREADMILL LOCOMOTION IN THALAMIC CATS. I. Belousov* and S. Romano*, CESTRO Neuro. Sci., Université de Montréal, Montréal, Québec, Canada H3C 1T7.

Previous work has shown that the proximal stump of cut dorsal roots (DR) are cyclically depolarized twice per cycle during fictive locomotion in spinal cats, with a greatest maximum during the activity of flexors and a second maximum during activity in the extensors. Single DR units discharging antidromically (AD) and rhythmically are often recorded in those cut roots. Rhythmic depolarisations can also be readily evoked by activation of peripheral afferents such as stimulation of the foot pads, we investigated whether the natural peripheral inputs during real locomotion could also play a role in the AD firing of DR units. Nine cats were decerebrated under Halothane anaesthesia. Locomotion was either spontaneous or triggered by MLR stimulation. Units were recorded from the proximal ends of the cut roots with Ag/AgCl electrodes in an oil pool. Representative EMGs of ipsilateral limb were recorded and the movements videotaped. The Table shows the distribution and characteristics of 73 labelled units out of a total of 92 recorded units. Their peak discharge may occur during swing or stance, they may fire in sync with their firing in stance or swing or be entirely confined within one phase (ON and OFF).

716.7 FDL AND SOLEUS MOTONEURONE ACTIVITY DURING SCRATCHING. S.H. Duque* and L. Castillo, CUCS, Universidad de Guadalajara and C.B., Universidad de Aguascalientes, Mexico.

This study analyzed weather the normal patterns of scratching activity of FDL and Soleus motoneurons that are known to be activated during scratching (FS). In normal cats, six hindlimb muscles were chronically implanted with patch electrodes for EMG recordings. In thalamic and later on, spinal cats, several hindlimb nerves were used for ENG recordings. The changes in resting potential (RP) in four FDL and five Sole motoneurones were also analyzed. In normal scratching cats, FDL-EMG exhibited tonic or phasic or intermingled activity, in contrast, Sole-EMG remain silent almost the entire episode of scratching. Occasionally occurred phasic Sole-EMG activity. In thalamic cats, FDL-ENG exhibited tonic, or phasic or intermingled activity. In 93% of the episodes of scratching occurred a sustained decrease in RP of FDL motoneurones. In 64% of the episodes, the sustained RP decrease was followed by phasic changes. At the onsets of the phasic bursts of activity occurred high frequency firing (doublets). Sole-ENG exhibited absence of motoneuron activity. Four out of five Sole motoneurones were hypoperalized during the episodes of fictive scratching. In spinal cats, FDL motoneurones only exhibit phasic activity but, without doublet. Sole motoneurones were phasically activated. Scratch generator produces phasic activity in FDL and Sole motoneurones, and supraspinal nuclei elicited sustained facilitation and inhibition in FDL and Sole motoneurones respectively.

716.8 IMMUNOHISTOCHEMICAL CHARACTERIZATION OF CAT SPINAL NEURONS ACTIVATED DURING FICTIVE LOCOMOTION. P.A. Carter*, B. Naga, A. Higgin, J. Don, D.M. Napier and I. M. Ross, Dept. of Physiology and Pathology, University of Manitoba, Winnipeg, MB, Canada R3E 0W3.

Anti-c-fos immunohistochemistry or intracellular injection of a 3000 MW tetramethylammonium-chloride depolarizing solution (TM-D) was used to label spinal neurons activated during fictive locomotion in paralysed, decerebrate cats. The immunohistochemical characteristics of c-fos-immunoreactive or TM-D labelled cells were compared using a variety of cytochemical markers. Locomotion-related spinal neurons were identified as TM-D containing the intracellulary-labelled cells subsequently processed using anti-choline acetyltransferase (AChT) and aspartate (AD). Populations of TM-D labelled lamina VII neurons were seen to contain either AChT- or aspartate-immunoreactivity. Simultaneous double labelling for c-fos and AChT, aspartate, calcium gene-related peptide (CGRP) or NADPH-diaphorase was carried out on non-TM-D labelled spinal cord tissue obtained from animals that displayed at least six hours of robust, MLR stimulus-evoked fictive locomotion. The majority of c-fos-immunoreactive cells were located in laminae VII and VIII of the lumbar spinal cord. Subpopulations of c-fos-immunoreactive neurons were seen to contain either NADPH-diaphorase reaction product, ChAT-immunoreactivity (ChAT), aspartate or to have close contacts with CGRP-immunoreactive fibres. These putative afferent inputs were found to be immunoreactive for certain transmitter systems such as glutamate, GABA and thyrotrophin releasing hormone. Supported by MRC Canada, Human Frontier Science Program and the Health Sciences Centre Foundation.

Using the lampry spinal cord in vitro preparation, we investigated the persynaptic plasticity control of neurons in lampry dorsal cells during fictive locomotion. The membrane potential of dorsal cells showed phasic depolarizations that occurred in phase with the ipsilateral ventral root burst. Negative current injections increased the amplitude of the depolarizations indicating that the depolarization is chemically mediated. In cells where no depolarization could be recorded, simultaneous intracellular recordings from their axons showed large depolarizations suggesting that the input synapses underlying the depolarizations are located on the axons. These depolarizations were insensitive to specific GABA_A (bicuculline) or GABA_B (phaclofen and saclofen) antagonists. On the other hand, GABA receptors are present on dorsal cells. GABA, muscimol and baclofen induced depolarizations in the dorsal cells which persisted after blockade of synaptically modulated transmission with tetrodotoxin. Specific GABA antagonists had no effect on the amplitude of the induced depolarizations, suggesting that the GABAergic effects on dorsal cells are not mediated by conventional GABA_A and GABA_B receptors. Small bipolar neurons containing GABA-ir and NPY-ir have close apposition on dorsal cells. Our evidence show that the synaptic transmission from sensory cells to the neuronal network generating locomotion, as previously shown for network interneurons (Alford and Grillner, J. Neurosci. 1991).


To clarify the mechanism for the generation of high-frequency oscillatory discharges (HFO) correlated among the inspiratory neurons, we have applied the in vitro preparation of perfused isolated brainstem developed by Morin-Sunur et al. (1992) to the newborn cats. One to 15 days-old cats were decapitated under pentobarbital anesthesia and the vascular system was perfused caudo-rostrally from the basilar artery with Krebs solution at 28°C. The afterdarts of discharges of facial (VII), vagus (X) glossopharyngeal (IX) and hypoglossal (XII) nerves were recorded simultaneously. By the spectral analyses, the following results were obtained: i) in the power spectra of the inspiratory nerve discharges, a clear peak with a modal frequency of 30.1±1.7 Hz (mean±SD, n=19) was consistently observed. ii) In the coherence spectra of any pair of these nerves recorded, a peak with high values of coherence (0.9±0.09, n=9) was consistently observed, demonstrating the synchronization of this oscillatory discharge between nerves. iii) A rise in the temperature of Krebs solution of the superfusion chamber significantly augmented the modal frequency of the oscillation. iv) Perfusion with a high K+ Krebs solution accelerated the respiratory rhythm but did not give any significant influence on the oscillation frequency. v) This synchronized oscillatory activity was observed unchanged in X, IX and XII even after ponto-medullary transection. Based on these observations, we conclude that the synchronization of oscillatory activity in the isolated brainstem of the newborn cats is identical to the HFO in mature animals and that the HFO could be generated in the medulla without any connection between other neural structures at least as early as ~12 hours after birth.

716.12 FAST OSCILLATORY ACTIVITY IN MESENCEPHALIC TRIGEMINAL NEURONS. C. Pedrosa*, I. Pope, J. Yamuy, P.K. Morales*, and M.H. Chase. Dep. de Fisiologia, Facultad de Medicina, Montevideo, Uruguay, and Dep. of Physiology and The Brain Research Institute, UCL School of Medicine, Los Angeles, CA 90024.

In contrast to all other muscle afferents, primary afferent fibers that innervate the jaw musculature have their cell bodies located in the central nervous system, in the mesencephalic trigeminal nucleus (MES-V). During experiments designed to study neurons within the reticular formation we found a remarkable oscillatory activity of the membrane potential of MES-V neurons, which is the object of the present report.

Intracellular recordings from MES-V neurons were obtained in the standard tissue slice preparation from rat brainstem; the patches were labeled by intracellular biocytin injection. The membrane potential of MES-V cells exhibited epochs of sinusoidal-like oscillatory activity within the range of 20-25 Hz; its amplitude was between 1 and 5 mV. Large oscillatory waves often triggered bursts of action potentials. The oscillatory activity was not observed at resting membrane potentials levels more negative than ~65mV, but it could be evoked by the injection of depolarizing currents. Tetrodotoxin abolished the oscillatory activity.

This is the first report of an oscillatory activity in the cell bodies of primary afferents. Consequently, we can only speculate on their functional significance. It is possible that this high frequency activity plays a role in the transmission of information from the jaw musculature to central neural structures. Because this activity originates in cell bodies, it is also possible that it may be synthaptically modulated during different behaviors. Supported by U.S.P.H.S. Grant NS 09999.

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717.1 INTRACELLULAR PATCH ELECTRODE RECORDINGS FROM NEURONS IN THE ISOLATED SPINAL CORD FROM NEONATAL RATS.

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The use of patch electrodes for intracellular recordings has several advantages compared to the use of traditional microelectrodes, particularly a better signal to noise ratio and the possibility for recording from small cells. We wanted to utilize these advantages in an intact spinal cord preparation, which has the benefit of intact large neurons and functional neuronal networks.

Neonatal rats (0.5-8 days old) were decapitated and the spinal cords dissected out with the preparation in ice-cold extracellular medium containing 0 mM Ca++. For the experiments, the cord was submerged in extracellular medium at room temperature, containing 2 mM Ca++. Pia mater could not be penetrated by the patch electrodes. Therefore, either a shallow cut was made from the ventral side immediately medial to the spinal cord, or a bennettized preparation was used. The patch pipette, with positive pressure, could then be lowered 100 to 500 mm into the spinal cord, and Deck seal formed by slight suction. This approach gave a high (>50%) success rate for stable (20 to 50 minutes) whole cell recordings, often with access resistance around 5 to 10 MO.

Recordings from a variety of cell types, visualized by biocytin, were made in the intermediate and ventral areas of the cord. Typical resting membrane potentials were -45 to -55 mV, and input resistances ranged from 20 to 90 nM amplitude. Cells could be recorded from during fictive locomotor activity, and the good signal to noise ratio made spontaneous and stimulated potentials from individual synapses clearly detectable.


Single channel activity was studied in inspiratory neurons identified by their pattern of firing related to phrenic motor output and used in the ventrolateral region (VLR) of the isolated in vitro brainstem-spinal cord preparation of a newborn rat. In outside-out recording under physiological condition, three channel types were identified by reversal potential, single-channel conductance, voltage sensitivity, pattern activity and tetrodoylammonium sensitivity. Two non inactivating potassium channels of 38 and 70 pS were found. Both channels showed voltage dependency, becoming more active at moderate depolarized potentials. The 70 pS channel showed tetrodoylammonium sensitivity at 0.1-0.4 mM. Also, a chloride channel of 25 pS was seen. These channel types may be involved in the control of the resting membrane potential and bursting activity of the inspiratory neurons and contribute to their functions in the mammalian brainstem.
T117.3

Discrete spinal rats respond to systemic injections of LOOPA or QUIPAZINE with coordinated locomotor activity on the ground, in water, or suspended in air (air stepping). However, LOOPA fails to elicit stepping in the hindlimbs of rats with mid-thoracic transections of the spinal cord, suggesting that the critical locus of action of LOOPA is in the brainstem. Alternatively, it is possible that LOOPA acts on spinal circuits, but that locomotor activation requires input from an additional spinal or spinal-motor system in the brainstem. In the present study we examined the possibility that LOOPA can elicit air stepping in the hindlimbs of spinal-transsected neonatal rats in the presence of the nociceptor agonist, capsaicin, intact neonatal rats and those that had received complete mid-thoracic (TS) transections of the spinal cord were injected subcutaneously with 0.1% LOOPA (100 mg/kg), QUIPAZINE (1.6 mg/kg), L-DOPA and quinpirole in combination, or the vehicle solution. Results showed that neither LOOPA nor QUIPAZINE alone elicited locomotor activity in the hindlimbs of rats with spinal cord transections. However, the combination of the two drugs elicited well-coordinated stepping in both the forelimbs and the hindlimbs, although the forelimbs and hindlimbs stepped independently of each other. In comparison to stepping of intact rats given LOOPA alone, the locomotor activity of the spinal rats receiving the drug combination stepped faster, and the hindlimbs slowed. The rates and topography of stepping were more variable in intact rats given the LOOPA/QUIPAZINE combination than in intact rats receiving LOOPA alone or in the hindlimbs of spinal-transsected rats. These results show that QUIPAZINE and L-DOPA must both act on spinal targets to produce locomotion. Supported by PhS grant NS28985 to DJS and OHV.

T117.5
LOCALIZATION OF THE CENTRAL PATTERN GENERATOR FOR HINDLIMB LOCOMOTION IN THE NEONATAL RAT IN VITRO. A LESION STUDY. O. Kjaerulf and O. Kiern (SPON: European Neuroscience, Neurophysiology, Dept. of Medical Physiology, Univ. of Copenhagen, Copenhagen 2200, Denmark).

Our previous studies with sufentanil have shown that laminae VII and X in the lower thoracic and lumbar regions of the spinal cord may be of possible importance for the generation of locomotion in the neonatal rat (Kjaerulf, Baranov and Kien, J. Physiol., in press, 1994). To test this conclusion we have monitored the rhythm-generating ability in surgically isolated segments of thoracolumbar segments of the spinal cord (Th1-Th9, Th12-L1). Rhythmic activity (RA) recorded from the ventral roots was induced with a combination of 5-HT and NDMA in spinal cords prepared from rats 2-2 days post-natal. Preparations consisting of segments Th12-L1 or L1-L3, while in preparations consisting of L4-L6 or L5-L6 either no RA or only slow, low-amplitude RA was found. This preparation from Th12 to L6 was sectioned in various planes. A midsagittal section did not prevent RA. Similarly, RA - alternating between the two sides - persisted after removal of the dorsal half of the spinal cord with a horizontal section. In contrast, preparations left from a paramedian section and comprising the lateral motorneuron pool, the lateral intermediate area and the dorsal horn showed no RA.

We infer that the neuronal networks important for spinal hindlimb locomotor activity predominate medially and ventrally in the thoracolumbar transition zone.

T117.7

Application of the glycine receptor antagonist strychnine to the bilaterally intact in vitro rat spinal cord transforms locomotor-like and alternating patterns of hindlimb motor activity into synchronous rhythms (Harder & Schmidt, Soc. for Neurosci. abstr. 1992). We have now applied whole-cell recording methods to examine excitatory inputs in homogenous motorneurons and interneurons during strychnine-induced synchronization. NDMA, serotonin and acetylcholine were used alone or in combination to induce baseline rhythmic activity. Following strychnine (1 μM), the rhythmic excitatory synaptic currents, in-phase and out-of-phase synchronous rhythmic ventral root activity, were recorded in all neurons examined. Rhythmic EPSCs (I_inh - 0 mV) as large as 200 pA resulted in synaptic drive potentials measured 50 mV in some neurons. The maximal EPSC amplitude in this cell occurred at +40 mV suggesting involvement of NDMA receptor activation. In one interneuron rhythmic synaptic activity was not observed, despite the injection of phasic ventral root activity by serotonin (10 μM) and NDMA (2.5 μM); however, the cell developed rhythmic 10 mV depolarizing potentials after addition of strychnine. Thus blockade of glycine receptors may result in the plastic recruitment of neurons not previously associated with the production of motor rhythm. Finally, although rhythmic inhibitory synaptic currents (I_inh - 50 mV) re-appeared in interneurons following removal of strychnine from the bath, ventral root activity remained synchronized. (Supported by HSCF and MRC)

T117.8
NMDA RECEPTOR-MEDIATED VOLTAGE OSCILLATIONS IN MAMMALIAN MOTOUNEURONS. S. Hochman*, L.M. Jorde, and B.J. Schmidt. Dept. of Medicine and Physiology, University of Manitoba, Winnipeg, Canada, R3H 0W3.

Whole-cell current clamp recordings were obtained from motorneurons in the bilaterally intact in vitro neonatal rat spinal cord in order to examine the effects of NDMA receptor activation. In one motorneuron, both application of serotonin (30 μM) and NDMA (10 μM) produced a progressive membrane depolarization accompanied by repetitive firing (6 Hz). Re-adjustment of the membrane potential to -60 mV, via intracellular current injection, resulted in the emergence of prolonged post-spike after-depolarizations (ADPs) peaking at 30 mV as well as an associated slowing of firing frequency (2 Hz). Following hyperpolarization of the membrane to -85 mV, Na+ spikes and ADPs were no longer present but rhythmic 10-15 mV slow-riasing membrane potential oscillations (4-5 Hz) persisted. These events were blocked by TTX (1 μM), 10-25 mV oscillations and occasional superimposed faster-rising high amplitude (30 mV) potentials remained. In another motorneuron, NMDA-induced membrane depolarizations induced a train of rhythmic post-removal oscillations that gradually decreased in amplitude (10-15 Hz). The oscillations were terminated by 200 nM TTX, after which the cell remained depolarized at -25 mV. Hyperpolarization of the membrane to -60 mV had no effect on frequency but the oscillations were blocked by 500 nM ZD 7288. The quality of oscillations remained the same whether or not the cell was subtested by TTX. Depolarization of the membrane to -60 mV and injection of a current ramp waveform revealed step changes in the membrane potential at membrane potential above 100 mV. However, the frequency of oscillations was insensitive to the concentration of NDMA in the bath. These observations suggest that mammalian hindlimb motoneurons are capable of endogenous oscillatory behavior in the absence of NMDA receptor activation. (Supported by HSCF and MRC/Canada)

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THALAMIC VPL IN RATS PROMOTES BEHAVIORAL RECOVERY AFTER SOMATOSENSORY LESIONS. J. Wells, S. M. Harry*, and J. M. Held*. Department of Anatomy and Neurobiology and Department of Physical Therapy*, University of Vermont, Burlington, VT 05405.

The transfer of information through VPL was found to be critical for the successful completion of a motor task and for the normal trajectory of the hindlimb. Bilateral VPL lesions resulted in a significant increase in the time to traverse a narrow bar for a reward and a significant modification of the swing phase of the gait cycle. The rats gradually decreased the MP-Phr coherence peaks had a frequency range of 39.1 - 66.4 Hz (mean 55.6 ± 8.3 SD Hz), with a mean coherence value of 0.55 ± 0.18 (range 0.38 - 0.80). The MP-Phr cross-correlogram patterns remained stable after the lesion, but the peak coherence peak decreased.


Muscle spindle afferents from the jaw closing muscles have their cell bodies in the nocosephalic trigeminal nucleus (VNoVn). Acoa connectives are sent to the trigeminal motor nucleus (VNvr), its border areas, and to the medial part of the oral nucleus of the spinal trigeminal tract (NVs). We report here on the firing properties of slowly adapting maseter spindle afferents recorded near the NVs during fictive mastication in urethane-anesthetized rabbits. Afferents were identified by their response to stretching of the jaw and to probing the muscle, and by short latency (<1ms) action potentials evoked by single pulse or repetitive (>30Hz) electrical stimulation of the motor nerve. Receptive field organization of the sensory motor cortex was used to evoke fictive movements that were monitored by recording the reticular activity in VNvr. The results show that the tonic firing of these afferents to a constant stretch is phasically inhibited during the opening phase, while an increase in frequency occurred during the closing phase of the cycle. Blocking of afferent inputs will allow the��ulation of the central orofacial function.

In 32 decerebrate, unanesthetized and paralyzed cats, we studied the characteristics of high frequency oscillations (HFOs) in MPs of medulla and E cells. Simultaneous recordings were taken from bilateral phrenic (Phr) and recurrent laryngeal (RL) nerves and from cells (0 - 1.5 mm caudal to obex, 3.2 - 4.2 mm lateral). HFOs during M recorded in 24 E and 15 I cells. In 14 of these were identified as RL cells (7 I, 7 E) by a sharp peak in the spike-RL nerve discharge correlation. The HFO in MP during I, superimposed on the discharge pattern of I cells, and hyperpolarization in I cells, developed gradually after onset, reached maximum amplitude around midline, and then gradually decreased. 39.1 - 66.4 Hz (mean 55.6 ± 8.3 SD Hz), with a mean coherence value of 0.55 ± 0.18 (range 0.38 - 0.80). The MP-Phr cross-correlogram patterns remained stable after the lesion, but the peak coherence peak decreased.

The thalamic VPL in rats promotes behavioral recovery after somatosensory lesions.
MUSCLE: ELECTROPHYSIOLOGY

V719.1

Previous studies (Haar Romeny et al., 1984; Segal 1982) have suggested three functionally discrete longitudinally oriented partitions within the biceps brachii long head (BBH). A recent pilot study from our lab (see English et al., 1990) suggested that the organization of BBH may be different than proposed by ter Haar Romeny et al. (1984).

In an attempt to clarify the organization of BBH, fine wire electrodes were inserted into 3 strips of BBH running from lateral to central in 6 subjects as determined from dissections. The activation order of motor units was determined for 3 tasks: elbow flexion, forearm extension, and shoulder flexion. In addition, recordings were made during elbow extension, forearm pronation, and shoulder extension, flexion and abduction. Spike-triggered averages (STA) were determined to determine the level of correlated activity. During both elbow flexion and forearm supination motor unit potentials were usually recruited first but were made second. Motor units from the central recording site were recruited more easily than the lateral site, especially when elbow flexion and supination were combined. However, motor units from the lateral recording site were most easily recruited during shoulder movements. Activity of motor units from the lateral site were not correlated with the motor units from the other sites, but motor units from the medial and central recording sites showed some correlated activity. This study supports partitioning of BBH, but possibly not as previously proposed. Future studies should study the activity of BBH during shoulder movements in more detail.

V719.2

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Spin--spin relaxation time (T2) measured from the magnetic resonance image of skeletal muscle layers linearly with activity of the muscle. T2 was used to investigate functional compartmentalization of the first dorsal interosseus (FDI) muscle of four healthy human subjects. Spin--echo images of two cross sections from the proximal half of the muscle were analyzed to determine regional differences in activity. Measurements immediately following flexion and abduction exercises of the left index finger. The exercise was against a force load that was 20% of the maximum work capacity. Each cross section of the muscle was divided into eight regions about the major axes of the muscle. In each cross section, the signal-to-noise ratio at four echo times (55, 50, 7.5, 100 ms) was measured and T2 relaxation time was calculated. Only areas showing homogeneous signals (i.e., no apparent high connective tissue or fat content) were measured. Percent change of T2 with muscle initial resting measures was used to quantify relative activation. The two major findings were: (1) during abduction, the dorsosmedial portion was more active (10.8% increase) than the ventrolateral portion (3.76%); and (2) during flexion, the middorsal and ventrolateral portions were more active (11.0% and 12.6% increases respectively) than the other portions (9.4%). These findings suggest that the relative activation of the FDI muscle is nonhomogeneous and varies with the task performed.

Supported by NIH grants AG 09000 and NS 20544 to RME.

V719.3
MOtor UNIT FIRING DURING SHORTENING AND LENGTHENING CONTRACTIONS. J.N. Howell 1, A.J. Fuglevand 1, M.L. Walsh 1, and B. Bigland-Ritchie 1.

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In order to examine motor unit firing patterns during movement, fine wire (25 µm) electrodes were inserted into the first dorsal interosseous (FDI). Unit activity was recorded as the FDI shortened and lengthened under constant loads and was compared to activity during isometric ramps. Single units, identified by wave shape analysis, could be followed reproducibly over many cycles of movement and in repeated isometric contractions. In most cases the first units to be recruited during shortening (concentric) were the last to be derecruited during lengthening (eccentric). An exception is shown, in which unit A turned off as B was recruited. B had a higher torque threshold during isometric activation than A. Unit activity was simultaneously decreased by a second motor unit (not shown), making it unlikely that the appearance of B in the trace shown represented an artifact of electrode movement rather than unit recruitment.

V719.5
ELECTROPHYSIOLOGICAL PROPERTIES OF DISSOCIATED PARAPODAL MUSCLE FIBERS FROM APLYSIA BRASILIANA. Paul J. Laurienti 1 and James E. Blankenship 1.


The pleurolophus, A. brasiliensis swims by rhythmic flapping of its bilaterally symmetric wing-like parapoda. Parapod motor neurons (MN's) and serotonergic motor neurons (PON cells) which innervate peripheral parapodal muscle are located in each pedal ganglion. PON cells and ergogeous serotonin (5-HT) have been shown to modulate the activity of the MN's and PON cells in vitro. The functional role of 5-HT on the parapodal MN's and PON cells has been explored. The presence of 5-HT in the parapodal MN's and PON cells has been confirmed. Current clamp experiments were performed with 3 types of parapodal MN's: one type is a tonic Mn that is activated by a constant current. The other two types of MN's are activated by a constant voltage command. The resting membrane potential (RMP) of the axons of the fibers is approximately -70 mV, which is close to 34 mV of the RMP of the MN's in the intact parapod.

V719.6
THIOKOTRIC BEHAVIOR OF THE PLANTARFLEXOR MUSCLES IN NORMAL INDIVIDUALS. Lamontagne A, Mahon T, Richards C 1, Teeney D. Physiotherapy Dept., Faculty of Medicine, Laval University and Neurology Research Center, Hopital de l'Enfant Jesus, Quebec City, Canada.

The primary aim of this study was to develop a method of assessing the thioxotrophy behavior of normal muscle utilizing a simple test that is dependent on the previous history of muscle contraction or length (thixotropy) thought to reflect intrinsic muscle fibers properties. We thus compared the effect of brief (5 sec) shortening and lengthening periods of the plantarflexors on the response of the plantarflexors during passive dorsiflexion (DF) of the ankle. Seven healthy persons (3 male, 4 female ages 25-30 yrs) were used. Force vs time (Fmax) curves between -23 to -8° of DF were measured at 20%/s with an isokinetic dynamometer (Kin-Com). Passive DF were made before (pre-test) and after (post-test) a 30 sec pulse in a lengthening of 80° position (condition 2) and a shortening of 80° (condition 1) for 10 trials at 20%/s. The last five trials of each test were averaged and the differences in the area (DA) under the torque-angle curves (-18° to 10°) between the pre-test and post-test were computed for each condition. In condition 2, the total area was decreased in all 7 subjects (DA = 1.94 Nm deg. -1 ± 1.11) while in condition 2, we observed a slight decrease for 6 subjects (DA = 4.82 ± 1.11), indicating that a decrease in torque responses are sensitive to the recent history of muscle lengthening. The Wilcoxon signed rank test computed between the DA for condition 1 and 2 indicated that the lengthened position induced a larger effect (p<0.01) on torque than the shortened position. These results indicate that 1) normal plantarflexor muscles exhibit a thioxotropic behavior that can be detected by the measurement of passive movement and 2) that the test may be applicable to the evaluation of changes in intrinsic properties of spastic muscle. (Supported by MRC of Canada).
T19.7
SPRINTED MOTOR UNITS SHOW TYPE-SPECIFIC RESPONSES TO CHRONIC ENLARGEMENT FOLLOWING PARTIAL DENERVATION OF RAT LATERAL GASTROCNEMIUS. E.L. SABURN AND P.F. GADINER*. Physiological Sciences, Univ. of Montreal, Montreal, Que., Canada H3C 3J7.

Motor units (MUs), enlarged subsequent to partial denervation may, with extended periods, diminish in their capacity for force production. We studied this issue by comparing normal lateral gastrocnemius (LG) MUs, isolated via splitting of the LS ventral root, with MUs which had been sprouted (14 section), for either 30 or 90 days. Compared to control, MU proportions were unchanged and overall motor unit twitch forces doubled at both 30 and 90 days. This increased tetanic force varied according to MU type: mean forces for S, FR and PF MUs were 315%, 128%, and 267% respectively, of controls. After 90 days FR MU force was further increased (25%), S was unchanged and FR decreased (26%), compared to 30 days. Twitch/tetanic ratios also responded differently according to MU type. Our results indicate that after the initial sprouting response is complete, MU reorganization continues based on size and/or activity level. Supported by NSERC Canada.

T19.9

A 7 degree-of-freedom (DOF) dynamic model of the human upper extremity has been developed for use in trajectory planning studies. Dynamic equations of motion were derived using Kane’s method and coded on a computer-graphics workstation. Included are 36 musculotendon forces to actuate the 7 DOF. Musculotendon origin and insertion points were obtained by dissection and measurement to +/- .1 mm in Cartesian space via an Osteopad digitizing probe and transformed to bone centered reference frames. Via points describing each musculotendon pathway were also computed where necessary to prevent the paths from penetrating bone surfaces during joint rotation, using the graphical display program SIMM. The model can be used to explore the neuromuscular strategies employed by the monkey to actuate joint angles when (i) holding the finger as a specified point in free space, or (ii) creating a trajectory in free space. When the finger position (or trajectory) is well within the physiological workspace, an infinite number of possible arm configurations will deliver the same endpoint position (trajectory). Optimal control theory was therefore used to compute minimum cost solutions to the kinematic redundancy problem. Cost functions include minimizing gravitational potential energy (Cg) and the sum of muscle stresses squared (Cg), which relates to metabolic energy expenditure. A pseudoviserous method was used to compute musculotendon stresses with minimum positional (trajectory) error. Preliminary results for the positional study indicate that cost function Cg predicts the shoulder angles, elbow flexion angle, and wrist flexion/extension angle, but cost function Cg does not perform as well. Supported by the NIH (NS 2675).

T20.1

The role of nitric oxide in cerebrovascular dilation during CSD is controversial. The purpose of this study was to examine effects of inhibition of nitric oxide synthase on cerebrovascular dilation during CSD. Since indomethacin pretreatment potentiates cerebrovascular dilation during CSD (Am. J. Physiol.: 269: R808-R810, 1995), we speculated that nitric oxide might play a larger role under these conditions. Urethane-anesthetized rabbits were equipped with a closed cranial window and pial arteriolar diameter was measured in vivo via intravital microscopy. Baseline arteriolar diameters ranged from 80-100 μm. We examined arteriolar responses to CSD in the absence of other drugs, and after intravenous administration of L-NNA (15 mg/kg) and/or indomethacin (10 mg/kg). CSD was induced by microinjection of 5% KCl. L-NNA reduced nitric oxide synthase activity by 90% in cerebral cortex (n=12 for controls and n=24 for L-NNA-treated) and reduced pial arteriolar diameter by 12%. In other animals, increased arteriolar dilation from 41 ± 5% to 76 ± 6% during CSD (p<0.05; n=8). In other animals, increased arteriolar dilation from 41 ± 5% to 76 ± 6% during CSD (p<0.05; n=8). However, administration of L-NNA in the presence of indomethacin failed to reductocclusion, following administration of indomethacin, inhibition of nitric oxide production by L-NNA fails to attenuate CSD-induced arteriolar dilation. Supported by HL 30260 and HL 46558.

T20.2

We have shown that cerebral blood flow (CBF) and cerebral vascular resistance (CVR) autoregulation at higher levels of arterial blood pressure (AP) after SAD in rat. In this study we sought to assess potential mechanisms for this phenomenon. CBF was monitored by laser flowmetry in intact rats in whom AP was increased by phenylephrine (PE), vasopressin, or angiotensin. AP was increased by PE in additional rats after: 1) SAD; 2) SAD plus bilateral removal of superior cervical ganglia (SCG); or 3) bilateral ligation of the renal artery and veins (ISN). We also examined the effect of unilateral SCG or bilateral SCG on CVR autoregulation under these conditions. AP was increased by PE in intact rats but did not break through at increased levels of AP in SAD rats with intact (15 ± 2 mmHg) or interrupted (16 ± 5 ± mmHg) sympathetic innervation of cerebral vessels. Breakthrough was seen in rats in which AP was increased with vasopressin but not in those treated with angiotensin, which blocked baroreflex-mediated bradycardia that occurs in intact rats. Breakthrough did not occur in SAD rats even when increases in plasma renin activity were prevented by isolation of the kidneys before SAD. This study suggests that the arterial baroreflex may participate in break through autoregulation at hypertensive levels of AP independent of its effects on sympathetic innervation of cerebral vessels or on plasma angiotensin levels. We speculate that break through of cerebral autoregulation in response to hypertension may result from release of an active vasodilator. Support: VA merit review and career award, AHA grant in aid, and NIH HL32205 and HL14388.

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THROMBIN INDUCES BRAIN EDEMA AND CONSTRUCTION IN CEREBRAL MICROVESSELS: POSSIBLE ROLE IN INTRACEREBRAL HEMORRHAGE.

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Thrombin is a naturally occurring protein involved in the clotting cascade. In addition to its role in thrombogenesis, it is thought to be involved in vasoconstrictive and inflammatory effects. While under normal conditions the level of thrombin in the brain is undetectable, during intracerebral hemorrhage (ICH) large quantities of thrombin are produced, leading to brain injury through thrombin. The present study presents a series of studies investigated the possibility that the intracerebral injection of thrombin induces local brain edema and microvascular constriction.

Rats received intracerebral injection of either vehicle, thrombin (1, 10, or 100 units), or a thrombin inhibitor (hirudin, 10 units; or Nω(O-2-aminophenyl)lysine (a-APL-DL, the hirudin mimic, naPAP), 0.2mM). Local brain water increased in rats injected with 10 or 100 units of thrombin (4.0% or 5.8% respectively; p<0.01 vs. sham animals). Animals injected with hirudin and hirudin had less water accumulation (2.0%; p<0.05 vs. thrombin alone). Similar results were obtained with the thrombin inhibitor, naPAP.

In order to investigate the possibility that thrombin may induce microvascular constriction, a second series of experiments was performed in which in vivo brain tissues were obtained from rats and examined with computerized videomicroscopy. Microvessels (30-70um in diameter) within the slices were monitored while the slices were superfused with thrombin (1 unit/ml in artificial cerebrospinal fluid) or vehicle (0.8% albumin solution) for 30 minutes. Thrombin induced a 35.4% microvascular constriction after 30 minutes, whereas treatment with vehicle resulted in a 2.9% constriction (p<0.005). These studies suggest that thrombin induces brain edema and that this effect may be due in part to the microvascular effects of thrombin. Brain edema/toxicity seen in ICH may be mediated by the local effects of thrombin. The results also suggest that the neurotoxic use of thrombin to augment hemostasis may be accompanied by local edema and vasocstriction.
721.1

NITRIC OXIDE FORMATION IS ESSENTIAL FOR ADAPTIVE MODIFICATION OF THE VEINULAR-OCULAR REFLEX, A MODEL FOR NEUROPLASTIC CHANGES IN THE GOLDFISH, Jan-Lu "Sheryl" S. Smith and James C. McIlrath 
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The gaseous second messenger nitric oxide (NO) implicated in neuroplasticity using in vitro models (Schuman and Madison, 1991), was tested for its role in adaptive modification of the veinular-ocular reflex (VOR) in the goldfish, as an in vivo model of neuroplastic changes. Two potential mechanisms for NO release in the cerebellum, activation of climbing fiber afferents (Southam and Garthwaite, 1989) and activation of non-NOS activity of astrocyte amino and receptors (Garthwaite et al, 1989), also occur during adaptation of the VOR. This reflex functions to maintain a stable visual image on the retina during head movement. Adaptive increases in VOR gain were induced by mechanical rotation of the head in the horizontal plane 180° out-of-phase with a visual stimulus. NO synthase (NOS) was first localized to goldfish cerebellum, as identified by NADPH-diaphorase staining. For the VOR study, NO production was pretreated by L-N^\text{N-monomethyl-arginine} (L-NMMA), a specific blocker of NOS, injected intracritically (i.c.) and after 14 shots, the response was observed. i.c. injection of L-NMMA also induced abolition of VOR adaptation. The inhibitory effect of L-NMMA application on VOR gain adaptation was not due to a non-specific action as assessed by laser Doppler flow measurements. Thus, these results suggest a role for NO within the cerebellum, in adaptive modification of the VOR, a learned response. (Supported by NIMHRO1 NS10455 and USDA CR 891003 to JLM)

721.2

MICRODIALYSIS WITH NITRIC OXIDE IN THE SEPTUM IMPROVES SOCIAL MEMORY OF RATS. T.Holen 1and M.Engelmann 2 The Scripps Research Institute, La Jolla, CA, U.S.A. 1Max Planck Institute for Psychiatry, Munich, Germany.

Nitric oxide (NO) synthase immunoreactivity in limbic areas of the rat brain including the septum implicates an involvement of NO in learning and memory. The present study tested whether NO in the septum of adult male adult rats is potent to modulate their juvenile recognition capacities. Animals which received artificial cerebrospinal fluid (ACSF) pregressed with a mixture of 5% NO in nitrogen via microdialysis into the septum showed a significantly improved juvenile recognition ability (p<0.01, ANOVA, n=13) compared to control sessions in which the same animal received ACSF pregressed with 100% nitrogen. To quantify the applied amount of NO, chemiluminescence detection was used to analyze the in-vitro release from the microdialysis probes showing that approximately 40 pmol NO per min were released from the probes into the surrounding medium. Blockade of endogenous NO synthesis by intraperitoneal administration of L-NAME (30mg/kg i.p.), however, failed to affect juvenile recognition in another group of rats (n=13). These results indicate, that neuronal substrate of olfactory memory located in the septum is sensitive to NO. However, the fact that blockade of endogenous NO synthesis did not affect the behavioral performance of other mechanisms may substitute for endogenous NO. Supported by the DAAD.

721.3


The role of nitric oxide (NO) as a neuromodulator in the CNS was assessed by a behavioral model of hippocampal-dependent learning. The dependent trait by inhibition of the NO-synthase (NOS) enzyme. Adult male rats of the Naples High (NH) and Low-Excitability (LX) lines, and of random-bred controls (NBR) were tested for 10 min in a L-maze during which two theta-related activity components such as walking and rearing, and an emotional index such as defecation scores were measured. Immediately upon completion of the test, the regional injection of the NOS inhibitor N^\text{N-nitro-L-arginine} (L-NOARG) 1-10 mg/kg or vehicle. Retention testing took place 7 days later. The results showed 10 mg/kg impaired long-term habituation (LTH) of the vertical component with prevailing emotional meaning, whereas both 1 and 10 mg/kg inhibited LTH of the defecation scores. In fact, 1 mg/kg exerted a small improvement in both strains. The disruptive effect of L-NOARG on long-term habituation of the vertical component in random-bred rats supports the hypothesis that NO may play the role of spatial anticipation and may improve the genotype-dependent differential effect of NO inhibition in NH and LX lines, compared to NBR-rats, strengthens their usefulness as a genetic model for altered spatial and non-spatial learning in the brain. (Supported by CNA 910304800 and Murast 40 grants).
1732.3 ORAL ADMINISTRATION OF L-NAME IN DRINKING WATER ALTERS WORKING MEMORY IN RATS. B.L. Cubas*, E.I. Brasil*, M.R. Hoss*, S. Dupel-Queiroz and S.A. Muccillo. Radiofrequency Radiation Division, Antrim Laboratory, Brookes Arn, TX 78234; *Depart of Biology, Trinity University, TX 78212.

In order to determine the role of nitric oxide (NO) in the maintenance of working memory of rats, the effects of oral administration of drinking water of the NO synthase inhibitor, N-nitro-L-arginine methyl ester (L-NMA), were studied. Substitution with a single test of recency-recall explored objects. Unlike other working memory tasks that require a single task to perform a reward such as food or water or to avoid shock, we tested spontaneous alternation of novel and familiar objects and it has been described as a pure working memory task (Masacari, 1988). Normal subjects spend a considerable amount of time in the environment with a single stimulus with which they have previous experience is presently available for the assessment of memory loss associated with the object. Memory changes were evaluated by measuring the relative time subjects explored familiar vs. new stimuli. Rats (n=15) that chronically drank an agonist 34.5% of 20 mg/kg/day L-NMA, spent significantly less time exploring a novel object than rats (n=15) that drank tap water (p<0.05). This effect of L-NMA was significantly attenuated by concurrent administration of L-Arginine (+50mg/kg/day). Total object exploration was not affected by our drug treatments, suggesting that our object discrimination task is not activity dependent. These data are consistent with the hypothesis that NO is required for normal functioning of working memory.


The acoustic startle reflex has a well described neural circuitry in which the nucleus reticularis pontis caudalis (RPC) plays an essential role. Nevertheless, the pharmacological reflex is not fully known. Local injection into the RPC of exocytotic amino acid (EAAs) agonists shows that glutamate may actuate startle at this level. EAA release at other synapses has been shown to enhance the effects of the second messenger cyclic AMP. Previous studies have demonstrated the involvement of the amygdala in the modulation of the startle response, particularly a CAMP-dependent mechanism of the RPC. As an initial test of this hypothesis, the present experiments evaluated whether a CAMP analog, 8-bromo-CAMP (BRC), or a phosphodiesterase inhibitor, Rolipram, would increase startle when infused directly into the RPC.

Rats implanted with cannulae in the RPC (PA-1.22, M.E. = +.15, D.V. = -9.6, at an angle of 20 degrees, a dose of 2.0 mg/kg/kg/day L-NMA, 0.15 MB of burst, 50 ms) were trained, at a 30 min. inter-trial interval, to a pre-drug baseline, and then induced successively with artificial cerebral spinal fluid (ACSF) and increasing doses of BRC (0.125, 0.25, 0.5, 0.75 and 1.0 μM). Immediately after each infusion they were tested with 40 startle stimuli. Other groups of animals were tested at above, but with 30 ms ventral of BRC alone or of BRC (0.25 μM). BRC produced a dose-dependent increase in startle amplitude. Repeated infusions of vehicle or 0.25 μM of BRC did not modify startle amplitude. Another group of animals infused with Rolipram 10 mg/kg/gal showed a marked increase in startle amplitude (281.3%). These results suggest that the CAMP second messenger system can modulate startle amplitude at the level of the RPC. To characterize more precisely the involvement of CAMP in startle modulation, we are currently examining the effects of other compounds known to alter CAMP activity. (Post-doctoral fellowship CNPq-Brasil # 20196093).

Erythropoietin is a glycoprotein produced endogenously in the kidney, which stimulates red blood cell production. We evaluated the effects of chronic treatment with recombinant human erythropoietin (Epoetin-α/Epo) on the performance of 8-month-old male C57BL/6J mice in a complex spatial task, the Morris water maze. Mice were treated with either Epo (1.5 units injected s.c. every other day) or vehicle (PBS also injected s.c. every other day). Results indicated that the treatment had no effect on maze performance after 9 weeks, but after 20 weeks the Epo-treated mice learned the task significantly faster than controls over 4 days of training as measured by mean distance (cm) to reach the goal platform. The results suggested that the improved performance in mice resulted from an increase in hematocrit which was not achieved until after 20 weeks of Epo treatment. Evaluation of several hematological parameters indicated that the only significant effect of Epo treatment was to raise hematocrit values at 20 wks. This rise was found to be significantly correlated with swim maze performance.


Ethanol blocks long-term potentiation in vitro (Sinclair and Lo, 1986) and inhibits NMDA-evoked activity of hippocampal neurons in vitro (Loving et al., 1989) and in vivo (Simon et al., 1993). In behavioral studies, acutely administered ethanol impairs the use of spatial memory (Matthews et al., RSA Meeting, 1994), and chronically administered ethanol impairs learning of a spatial task (Arendt et al., 1988) that has been shown to be sensitive to hippocampal damage (Olton and Papas, 1979). More recently, Matthews et al. (RSA Meeting, 1994) report that ethanol, but not diazepam or MK-801, markedly altered the spatial properties of hippocampal neurons recorded electrophysiologically in awake behaving rats. In contrast to these results, however, Devenport et al. (1989) conclude that ethanol is virtually without influence on spatial localization. In the present study, we investigated the effect of ethanol on spatial and non-spatial tasks that Matthews and Best (1993) demonstrated to be differentially sensitive to fimbria/ fornix lesions. Results show that animals injected with ethanol prior to each training session learned significantly slower than saline-injected animals on the spatial task (p < 0.1). In contrast, ethanol-injected and control animals performed similarly on the non-spatial task (p > 0.1). These results suggest that ethanol produces an impairment in spatial learning similar to that produced by fimbria/fornix lesions.


We demonstrated that Nifedipac (NEF) ameliorates eyelock classical conditioning (ECC) in older rats in the 750 ms delay paradigm. The present study is an attempt to identify the brain site of action. If NEF affects ECC via the hippocampus, injection of NEF in rabbits with intact hippocampus should ameliorate ECC whereas injection in hippocampectomized rabbits should not. Five groups of older rabbits were run in 750 ms delay ECC: 1) hippocampectomy, 10 mg/kg NEF (N=8); 2) partial cortical removal, 10 mg/kg NEF (N=9); 3) sham surgery, 10 mg/kg NEF (N=9); 4) no surgery, 10 mg/kg NEF (N=8); 5) no surgery, vehicle (N=8). Among groups treated with NEF, trial 2 learning criterion (T/C) was significantly greater in hippocampectomized rabbits (1,038) than in rabbits with intact hippocampus (cortical = 738; sham surgery = 445; no surgery = 473). Rabbits with vehicle had significantly more T/C than those with NEF (probability of percentage of conditioned response responses supported the results with T/C). Hicccupampcromized rabbits with NEF learned more slowly than cortical surgery and controls with intact hippocampus. NEF may act to ameliorate ECC in older rabbits via the hippocampus. Supported by Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan.

EFFECT OF AIT-082 ON ETHANOL-INDUCED AMNESIA AND BEHAVIOR. R.P. Ritzmann*, P. Prieto-Gonzalez, C.L. Melichar, F. DeLeon-Jong, and J. Glicky. Olive View/UCLA Medical Center, Sylmar, CA 91342 and Advanced ImmunoTherapeutics, Tustin, CA 92680

AIT-082, a derivative of the purine hypoxanthine, has been shown to improve memory in the wls-shift test of working memory. This test consists of placing a mouse in a T-maze with a reward in both goal boxes; the mouse is allowed to choose one of the goal boxes. On the next trial, if the mouse remembers which box it entered on the previous trial, it will enter the other box. By increasing the delay between trials, the length of time a mouse can remember can be determined. In C57BL/6 mice, the longest delay that these mice can remember is 120 seconds. Mice receiving AIT-082 (30 mg/kg) were able to remember at delays over 180 seconds. Ethanol (0.5 mg/kg), 10 minutes prior to testing, at 120 second delay, reduced the correct responses by 60%, without altering the time to leave the start box or the time to traverse the maze, indicating that ability to perform was not impaired. AIT-002, given 20 minutes prior to ethanol, blocked the amnestic effect of ethanol.

A behavioral analysis of the effects of AIT-082 was performed at doses from 0.005-60 mg/kg. The tests included: locomotor activity, exploratory activity, plus maze test of anxiety, hot plate analgesia and roto-rod test of motor coordination. The only significant effects of AIT-082 were improved performance on the roto-rod test at doses from 0.05 to 60 mg/kg and a decrease in locomotor activity only at the highest dose (60 mg/kg). The decrease in locomotor activity along with the lack of any effect on exploratory activity may be due to the fact that these tests would be consistent with the effects of AIT-082 on memory in other tests. Supported by grants from NIH and NIA (AG099911).


Ethanol blocks LTP in hippocampal slices (Sinclair & Lo, 1986) and inhibits NMDA-evoked activity of hippocampal neurons in vitro (Simon et al., 1993). Chronic ethanol also impairs learning a spatial task (Arendt et al., 1988) that has been shown to be sensitive to hippocampal damage (Olton & Papas, 1979). In addition, Matthews et al. (see accompanying abstract) report that ethanol alters the spatial properties of hippocampal neurons. We investigated the effects of acute ethanol on two tasks requiring spatial memory and one requiring non-spatial memory. Ethanol significantly reduced the ability to use spatial memory but not non-spatial memory. Also, MK-801 (only at doses that produce ataxia) but not diazepam impaired spatial memory. Neither drug affected non-spatial memory. Therefore, ethanol's contribution to auto accidents, may not be limited to impairment of judgment and reflexes but may include diminished cognitive necessity for the use of spatial memory.

BENEFICIAL EFFECTS OF PRE-084, A SELECTIVE δ LIGAND, ON PHARMACOLOGICAL MODELS OF LEARNING IMPAIRMENT IN MICE. T. Maurizio*, T.P. Su*, D.W. Pavlich* and A. Prival1. 1 INSEEM U 336, INSECM, 8 rue des Ecoles Normales, 14053 Montpellier cedex 1, France; 2 NIDA-Addiction Research Center, NIBL, POB 5180, Baltimore, MD 21224. 3 Bio-CAD Corporation, 545 Oakway Park, Sunnyvale, CA 94086.

We investigated the effects of PRE-084, a selective sigma ligand derived from phenylcycloide, on several pharmacological models of learning impairments in young adult mice. Tests included spontaneous alternation in a Y-maze, for immediate spatial working memory, and the step-down type passive avoidance, elevated (+) maze, and place learning in a water maze, for long-term memory. (1) PRE-048, at doses about 1 mg/kg sc., significantly attenuated the impairment of alternation, decrease in step-down latency, and increase in transfer latency induced by MK-801 (0.2 mg/kg ip). These effects were antagonized by the simultaneous administration of BMY-14802 (10 mg/kg ip), and suppressed in mice chronically treated with haloperidol (4 mg/kg/dy sc for 7 days). (2) At the same dose range, PRE-084 significantly attenuated the impairments induced by mecamylamine (10 mg/kg ip), but not by scopolamine (1 mg/kg ip). (3) PRE-084, at 0.3 - 1 mg/kg, also prevented the impairment of alternation, decrease in step-down latency, and impairment of place learning induced by the calcium antagonist nimodipine (0.3 mg/kg ip). These results confirm previous studies showing that sigma ligands may modulate the learning processes, mediated via the NMDA receptor or the nicotinic cholinoreceptor activation, and the resulting calcium mobilization. Thus, PRE-084, by acting selectively at sigma sites, may be useful in improving the cognitive impairments due to aging or Alzheimer's disease.
THE NOVEL COGNITION ENHANCING AGENT S 12024-2 FACILITATES OBJECT AND SOCIAL RECOGNITION MEMORY AFTER ACUTE OR CHRONIC TREATMENT IN RATS. W. Fassbinder, J. Pickart, A. Schöffstetter, and J. Joles, Dept. of Psychiatry and Neuropsychology, Univ. of Limburg, Maastricht, NL-6200 MD, and Dept. of Pharmacology, Univ. of Amsterdam, The Netherlands.

Preliminary experiments suggested that S 12024-2, a novel compound [Sever], might enhance memory. We used two different tests in establish whether S 12024-2 facilitates recognition memory. Twenty adult male Wistar rats were tested in the object exploration test [Ennaceur & Dellacour, Behav. Brain Res. 1988]. In the first trial (3 min) exploration times were scored for two identical objects. After 24 hr the rats did no longer discriminate between an identical, familiar object and a novel object. When they had received S 12024-2 (3.4 mg/kg i.p) immediately after the first trial, they discriminated between the two objects indicating that the treatment facilitated recognition memory. This effect of S 12024-2 was replicated. Subsequently, two groups were formed that received either distilled water (n=8) or a solution of S 12024-2 (16 mg/kg/day, n=12) to drink. After 10 days the S 12024-2-treated, but not the control, rats showed significant recognition of the familiar object. This effect was replicated after 17 days of treatment.

Another group of 20 rats was used for the social memory test [Danziger et al., Psychopharmacology, 1987]. A similar design was used: all animals were tested after post-training ip injection of saline or 3.4 mg/kg S 12024-2. After 24 hr a hour in exploration of a juvenile rat was found only when treated with S 12024-2. This effect was replicated. In the chronic treatment condition S 12024-2 facilitated social recognition after 6, 10, and 12 days of treatment. From these results it can be firmly concluded that S 12024-2 is effective as a cognition enhancing agent with respect to recognition memory. (Supported by J.R.S., Courbevoie, France.)


Two studies were conducted to determine the lowest dosage of phenobarbital (POB) that could (1) maintain discriminative control in a drug discrimination (DD) task, and (2) produce appreciable state dependent learning (SDL). Both studies progressed through successive cycles of training in a 2-lever task with a different dosage used in each cycle. Both used Long Evans male rats and injections of phenobarbital (POB) or saline (S). The SDL study used the following procedure: (1) Train for 5 sessions while D or S is present; (2) Give one D and one S test session (ever lever reinforced); (3) Train for 5 sessions to lever 2 while N (or D); (4) Give two D and two N test sessions; (5) Untrain the rats by reinforcing response on the drug-inappropriate lever. SDL was noted after D->S-N transitions with doses as low as 3 mg/kg. The DD study used conventional D vs N training with FH10 vs extinction schedules of reinforcement on the correct and incorrect lever. Doses during successive cycles of 18 sessions was selected by a dosage titration paradigm to determine the lowest dosage that could support above-criterion discriminative control. The threshold irregularly-discriminated dosage was 3 mg/kg and extrapolation from the accuracy vs. dosage plots indicated an absolute threshold dosage of about 2 mg/kg. Hence SDL generalization failures occur with dosages almost as low as the lowest dosage that can be discriminated after prolonged DD training. The results suggest that "drug cues" may not be processed via mechanisms that allow the rats to "pay to attention" to drug effects.

CIRCADIAN CLOCK REGULATES ROD AND CORE INPUT TO GOLDFISH CORONAL HORIZONTAL CELLS. S.C. HangeI and T. Wang, Dep. of Ophthalmology, University of Alabama School of Medicine, Birmingham, AL.

To determine whether a circadian clock regulates the effects of dark adaptation in the retina, goldfish T-type corona horizontal cells (HCS) were studied. Fish were maintained in constant darkness. Surgery was performed under dim red or infrared light. Retinas were superfused with darkness for 90 min (prolonged darkness®), following which a HC was impaled without the aid of any light flashes. Following prolonged darkness during subjective day, light responses were fast and response amplitudes averaged 17 mV to the brightest stimuli. Spectral sensitivity was similar to that of red (625 nm) cones. During subjective night, light responses were slow, response amplitude averaged 2.5 mV to the brightest stimuli and response duration was 5-6 times longer than stimulus duration. Spectral sensitivity was similar to that of red HCs. Reversing the light-dark cycle for 10 days reversed the rhythm of dark adaptation. These results indicate that a circadian clock in HC light responses such that following dark adaptation, red cone input predominates during the subjective day and rod input predominates during the subjective night.


We examined the effects of acute post-training injections of estradiol-hydroxypropyl-β-Cyclodextrin inclusion complex on rats trained in a single-platform spatial water maze task. Unlike traditional forms of sex hormone administration, injection of estradiol-inclusion complex results in a rapid rise of hormone in blood plasma, similar to that induced by normal hormone release. Rats received a single 8 trial (30 second inter-trial interval) training session with a submerged escape platform located in the same quadrant of the maze on all trials. Following trial 8, rats received a post-training subcutaneous injection of estradiol (0.25, 0.5, or 0.75 mg/kg) or saline. On a retention test session 24 hours later, latency to mount the escape platform was used as a measure of memory. The retention test escape latencies of the animals given 0.75 mg/kg of estradiol were significantly lower than those of saline treated rats, indicating an enhancement of spatial memory. Estradiol at doses of 0.25 and 0.5 mg/kg had no effect on retention test latencies. The findings indicate that acute post-training administration of estradiol can enhance spatial memory in male rats.


Wistar rats were exposed to two lighting conditions, constant light or a standard 12/12 hr light/dark cycle. The animals were acclimated to lighting conditions for 5 wks prior to ethanol (EtOH) acquisition with water and food available ad lib. EtOH was presented in increasing concentrations from 2% (v/v) 95% EtOH with water) to 10% on alternate days in free choice with water. Immediately following the acquisition phase, a maintenance period was initiated which began with everyday presentations of 10% EtOH solution in free choice with water. After 10 days maintenance, lighting conditions were switched such that rats in constant light received EtOH and rats in subjective day, light responses were fast and response amplitudes averaged 17 mV to the brightest stimuli. Spectral sensitivity was similar to that of red HCs. Reversing the light-dark cycle for 10 days reversed the rhythm of dark adaptation. These results indicate that a circadian clock in HC light responses such that following dark adaptation, red cone input predominates during the subjective day and rod input predominates during the subjective night.


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BIOLICAL RHYTHMS: LIGHT
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**TH22.3**


Hamster PLI or ENK-IR neurons in the IGL project to the suprachiasmatic nucleus (SCN) by a geniculo-hypothalamic tract (GHT). Cells also project to the contralateral IGL, but few contain NPY- or ENK-IR. A major third IGL-ef fluent path is now identifiable.

NPy- and ENK-IR fibers extend dorsally from the IGL into the superior thalamic radiation. A thin, vertically oriented terminal field is evident in the PLI along the lateral border of the anterior pretectal nucleus, extending ventrally and laterally along the medial geniculate nucleus. Retrograde studies (cholera toxin β-IR) show IGL cells projecting to the dorsal thalamic and pretectal nuclei. Anterograde transport of Phaseolus vulgaris lectino-agglutinin (ENK-IR) with the suprachiasmatic network (SCN) and the lateral pretectal and pretectal nuclei. The effect of the clock on phase shift, we administered an NP4 just prior to a light pulse at CT 18. Hamsters housed in constant darkness (DD) were implanted with a guide cannula aimed at the suprachiasmatic nucleus. Animals were injected with 200 ng of NPY or 200 ng of normal serum (NS) at CT 18 under a dim red safe light. They were then either exposed to light (approximately 200 lux) for 15 minutes or returned to DD. Hamsters shifted significantly more to the light pulse when pretreated with an NP4 (mean NS + light = 1.18 minutes; mean ANP + light = 1.92 minutes; p < 0.01 on both two-tailed paired t test and Wilcoxon test). Therefore, antiserum to NP4 did not block photic advances and in fact potentiated them. This indicates that the low levels of NPY found in the SCN at this time may be involved in photic phase shifting.

**TH22.5**

PHASE SHIFTS TO LIGHT AT CT18 ARE INCREASED BY PRETREATMENT WITH ANTISERUM TO NEUROPEPTIDE Y. S.M. Bliello, N. Mysogoyev and M. Ralph, Deps. of Ecology and Psychology, University of Toronto, Toronto, Ont., Canada M5S 2A.

Previous work has indicated that antisem to neuropeptide Y (NP4) blocks phase shifts to certain non photic stimuli at CT 4. To insure this effect was specific to non photic phase shifts, we administered an NP4 just prior to a light pulse at CT 18. Hamsters housed in constant darkness (DD) were implanted with a guide cannula aimed at the suprachiasmatic nucleus. Animals were injected with 200 ng of NPY or 200 ng of normal serum (NS) at CT 18 under a dim red safe light. They were then either exposed to light (approximately 200 lux) for 15 minutes or returned to DD. Hamsters shifted significantly more to the light pulse when pretreated with an NP4 (mean NS + light = 1.18 minutes; mean ANP + light = 1.92 minutes; p < 0.01 on both two-tailed paired t test and Wilcoxon test). Therefore, antiserum to NP4 did not block photic advances and in fact potentiated them. This indicates that the low levels of NPY found in the SCN at this time may be involved in photic phase shifting.

**TH22.7**

EFFECTS OF PROTEIN SYNTHESIS INHIBITION ON PRESUMPTIVE OSCILLATOR PROTEINS AND CIRCADIAN PHASE OF THE OCULAR CIRCADIAN PACEMAKER OF BULLA GOUUDIANA, Michael M.

Roberts* and Nancy A. Krueger, Dept. of Biology, Clarkson University, Potsdam, NY, 13699-5806.

The eye of the marine snail, Bulia goudiana, contains a circadian pacemaker. In previous work we have proposed that members of two protein families, some of whom regulate the wukayrtoc cell division cycle, are involved in circadian rhythm generation. Using Western blotting techniques we have identified a protein (p40) possibly related to the cell division kinase, p34cdk, in the B. goudiana eye. We have also identified a presumptive 65kDa homolog (p66) of the regulatory protein cyclin.

The current work we have characterized the phase dependent effects of the protein synthesis inhibitor cycloheximide (CHX 10μM) on both the phase of the circadian rhythm and the levels of p40 and p66. CHX had no effect on the rhythm when given at CT -4, while phase delays appeared at CT 8-10 (1.6 ± 0.75 hrs) and increased gradually to 3.0 ± 1.7 hrs from CT 22-4. In preliminary studies, p40 levels increased by CHX during the day and decreased at night. In contrast, CHX increased p66 when given from CT 18-22 and decreases p46 at other phases. The effects of CHX on circadian phase as well as on the level of these proteins are consistent with our hypothesis that p40 and p66 may be involved in circadian rhythm generation.

**TH22.8**

AFFERENT COMPONENTS IN THE PROTOCEREBRAL (pc) CIRCADIAN SYSTEM OF CRAYFISH, B. Barrera-Mera* and E. Barrera-Calva, Dept. de Fisiologia Fac. de Medicina UNAM, A. Postal 70-250 Mexico 04510 D.F.

The pc system of crayfish (Fig 1) has been studied in isolated preparations "in vivo". Under this condition a robust circadian (cr) rhythm of sustaining response neurons (s), retina (r) and distal (d) retinal shielding pigments (RSP) shine its endogenous character. While this rhythmic activity can occur spontaneously, usually, presumably, in the normal animal, the pc c pacemaker (pm) is modulated by interneurons input from the rest of the nervous system, as shown by the variety of elements in the optic tract. In the pc c system we found that only the c response of r, s and the jittery fibers remain unchanged. They apparently are the afferent input of both left and right pc c pm, which are expressed throughout the synchronous release of neurochemical neurotransmission as light-adapting hormone (LATH) from the sinus gland (sg).
BIOLOGICAL RHYTHMS: LIGHT 1767

723.1

USE OF LED TECHNOLOGY IN PROVIDING CONTROLLED LIGHTING FOR ANIMAL RESEARCH. M. Drews, I.-H. Tang, T. Tandon, and C.A. Fuller. Section of Neuroscience, Physiology & Behavior, University of California, Davis, California 95616-8519.

The light environment is critical for animal research, including both basic husbandry and experimentation. In order to study how animals respond to different aspects of light, we utilize the characteristics of light emitting diodes (LED). The LED technology offers several advantages, including: 1) they are sold state devices, 2) they provide narrow spectral band output near their peak wavelength, 3) they are available in several discrete wavelengths, 4) they demonstrate negligible shift in spectral distribution at different intensities, 5) they have a high degree of stability and longevity, 6) they produce virtually no heat, 7) they are small and require minimal room requirements, and 8) their output intensity can be controlled linearly and dynamically. We have developed an autonomous computer based controller to provide variable profile light cycles, including: 1) rate of light intensity change, 2) range of minimal and maximal intensity, and 3) duration. The output intensity profile can be monitored in real time. We have examined the photic entrainment of hamsters in a simulated natural light-dark cycle using this device. This device can also be applied to study the photic entrainment thresholds of a defined spectrum. We have also studied the entrainment behavior of a small primate, the squirrel monkey, using the narrow spectral qualities of the LED light source. (Supported by NASA Grant NAGZ-792.)

723.2


This study compared the entrainment characteristics of simulated natural light-dark (LD) cycles and square wave LD cycles (14:10) in golden hamsters. The simulated natural LD cycle was composed of a 4 h linearly ramped dawn-dusk-mimicking period, a 6 h full light intensity day period and a 4 h linearly ramped dusk-dawn-mimicking period. Animals were implanted with biotelemetry transmitters to record body temperature and activity at 10 min intervals via microcomputer. The phase angle at which animals entrained was determined by onset times of the animals' ambulatory activity. Animals under simulated natural LD cycles exhibited advanced relative to the LD cycles. An additional test was performed to examine the role of bilateral vs. unilateral eye in the entrainment responses. Animals received unilateral optic nerve transection exhibited a further degree of advance activity onset. In all conditions, there was a stable phase angle between body temperature and ambulatory activity under simulated natural LD cycles, suggesting anticipatory behavior. Preliminary data also demonstrated that animals with a unilateral optic nerve transection showed different c-Fos expression compared to intact animals in response to identical light pulse stimuli.

723.3

A MONTE CARLO TECHNIQUE FOR CHARACTERIZING EPOCHS OF ELECTROCOROLLARY ACTIVITY IN THE FETAL SHEEP. S. B. Robinson2, W. P. Smulovitz1, G. H. Wong1, S. S. Robertson2, and P. W. Nathaniel1. 1Laboratory of Perinatal Neurotoehy, Dept. Psychology, Binghamton Univ., Binghamton, NY 13902; 2Dept. Human Development and Family Studies, Cornell Univ. - Laboratory of Pregnancy and Newborn Research, College of Veterinary Medicine, Cornell Univ., Ithaca NY 14853.

Electrocorollary activity (EcOG) recorded from fetal sheep is a measure of sleep-wake state that differentiates into epochs of low voltage and high voltage late in gestation (term = 145 days). Five fetal sheep were surgically prepared at 113-114 days of gestation with an array of chronic instrumentation, including stainless steel electrodes overlaying the dura of the parietal lobes to measure fetal electrocorollary activity. EcOG signals were sampled at 32 Hz, amplified, full-wave rectified, averaged over 1 s intervals, and recorded continuously over days 121-133. Brief epochs of low- and high-voltage EcOG were distinguished on day 121, with durations that persisted significantly longer than predicted by Monte Carlo reordering of EcOG time series. Average EcOG voltage increased with age, and duration of low- and high-voltage EcOG epochs also increased, with the most rapid development evident about days 129-131. The developmental increase in duration appeared to be due to the selective advancement of the (c-5a) transitions in EcOG activity that interrupted epochs of longer duration. These results suggest an objective, quantitative strategy for characterizing epochs of low- and high-voltage electrocorollary activity that may be relevant for understanding the prenatual ontogeny of behavioral states.

This research was supported by a subcontract with Cornell University to WPS and SBR under NIH Grant HD 28014.

723.4

EVIDENCE THAT MAMMALIAN CIRCADIAN RHYTHM IS COMPOSED OF ULTRADIAN CYCLES AND SIMILARITIES BETWEEN CALCIUM OSCILLATION IN SUPRAECHASMATIC NUCLEUS CELLS TO THE MAMMALIAN CIRCADIAN PACEMAKER. Shinnazamaki1, Shin-Ichi T. Inouye2 and Yoichi Kuroda,1

1Department of Molecular and Cellular Neuroscience, Tokyo Metropolitan Institute of Gerontology, 2-5-1 Mutohida, Fuchu-shi, Tokyo 183, Japan.; 2Laboratory of Integrative Brain Function, Mibishi Kasei Institute of Life Sciences, 11 Minami-Ooya, Machida-shi, Tokyo 194, Japan.

A central question in the elucidation of the mechanism of circadian rhythm generation in the suprachiasmatic nucleus (SCN) is whether individual cells have an intrinsic capacity to generate circadian rhythms or the rhythmicity requires the interaction of cells. Like cortical neurons in culture (Kuroda et al. Neurosci. Lett. 125: 185, 1992), dissociated cultures of rat SCN show spontaneous oscillations of intra-cellular Ca2+ with 5-8 second period (ultradian rhythm). Replacement of Mg2+ by deuteron oxide (D2O) in the culture medium lengthened the period of this oscillation, while tetrodotoxin did not inhibit the deuteron oxide sensitive Ca2+ oscillation. Since these characteristics resemble those of the mammalian circadian pacemaker, the ultradian Ca2+ rhythm may be involved in the circadian rhythm or have a similar rhythm generation mechanism. The gap junction inhibitor, halothane, stopped this oscillation. Gap junctions may be necessary in order to generate rhythmic oscillations.

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272.5
THETA EEG GENERATOR IN RAT NECORTICAL BRAIN SLICES. H.S. Lukatch and M.B. MacEchern. Dept. of Anesthesiology, Stanford University School of Medicine, Stanford, CA 94305.

The present study describes a novel neocortical EEG theta generator in rat brain slices. Theta EEG frequencies (7.31 ± 0.94, 419) were evoked in vitro by mimicking ascending cholinergic and GABAergic tone with carbacol (100 μM) and bicuculline (10 μM). Mapping studies revealed that this theta activity was limited to a strip of medial cortex running rostral caudally (partially overlapping cortical areas O2/CMM, RSA, Fr1, and Pr2). The presence of a theta generator intrinsic to cortical areas O2/CMM and RSA was confirmed by isolating mini-slices of these areas and inducing theta EEG activity in these mini-slices. Two electrode differential recordings were used to determine that theta generating cells were localized to layer 5. This theta activity was blocked by the muscarinic receptor antagonist atropine sulf ate (0.5 μM), similar to in vivo cholinergically driven theta. Preliminary loose patch single unit recordings revealed two cell types which exhibited different firing patterns in relation to the theta oscillations. One cell type fired only at the beginning of each theta burst and remained silent during the theta train. The other cell type was quiet at the beginning of each theta train, and attained its highest rate of discharge during protraction.

Supported by USAF OSR/SCREE Graduate Fellowship.

272.6

Temporal fluctuations which cannot be explained as consequences of statistically independent random events are found in a variety of physical and biological systems. The fluctuations of these systems can be characterized by the power spectrum density S(ω) decreasing as ω^-k at low frequencies with an exponent 0.5 ≤ k ≤ 1.5. We present a new model to describe the individual biological rhythms of humans using data from a colleague who has kept daily records for four years of his state of well-being applying a fifty-point magnitude category rating scale. This time series (S(t)) was described as a point process by introducing discriminating rating levels r and for the occurrence of r(t) > r (ups) and r(t) ≤ r (downs). R(t) after introducing a certain r or r is described by ρ (t) = ∫ P(t) dt, where P(t) is the probability density function of the random variables t_j, the time of a particular value R(t) ≥ r or R(t) < r. Applying counting statistics (Knuffke et al. Fraktale 1,800,1993) to evaluate S(ω) the following scaling laws were found: first scaling region 1 ≤ S(ω) ≤ 15d for the 'ups' and 'downs' where S(ω) = ω^(-k) with k = 0,7; second scaling region R(t) ≥ 15d exhibiting an almost random behavior with k = 0.0.

We speculate that the basic mechanisms of the neuronal/basal systems responsible for biological rhythms are expressions of a 'self-organized critical state' as introduced by Bak, Tang and Wiesenfeld (Phys. Rev. Lett. 59:381,1987) for physical systems. Because of the first scaling region and based on one's own monitored biological rhythm it should be possible to predict future episodes (Δ t ≤ 15d) with a certain probability by using methods of nonlinear time series analysis or modified feed-forward neural networks learning with backpropagation algorithm.

Supported by the Peter Bente Helfer-Stiftung in the Stifterverband für die deutsche Wissenschaft.

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274.1
NEONATAL ISOLATION ENHANCES LTP AND RESPONSES TO AMPHETAMINE CHALLENGE IN JUVENILE RATS. J. Hoffman, A. Arabahi, P.J. Austen & A. Brassett. Neuroscience Program, Trinity College, Hartford, CT 06106.

Using a within-litter design, pups were isolated from mother and littermates for 1 hr. per day over PN days 2-9. At 30 days of age, the response of the dentate granule cell population to tetanization of the perforant pathway was examined in previously isolated and non-isolated littermates. Measures of population spike amplitude (F1) and population EPSP slope obtained at several following tetanization were used to assess the magnitude and duration of LTP obtained from each group. Preliminary results indicate that the degree of enhancement to both EPSP slope and F1 measures obtained from neonatally isolated animals was markedly greater than that of controls across the first 1 hr. recording period. In a separate group of littermates, striatal dopamine response to amphetamine (7.5mg/kg) challenge was assessed on PN day 27 by in vivo microdialysis. Controls treated, prenatally isolated, animals showed a significantly higher dopamine levels over the first hr. following amphetamine administration. Behaviorally, these animals demonstrated a significantly enhanced amphetamine hyperactivity. In summary, neonatal isolation significantly enhances LTP and amphetamine-induced behavioral and dopaminergic responsivity. The results suggest that the impact of neonatal isolation on response properties of both the hippocampus and striatum endures into the juvenile period of development and can be revealed by both electrophysiological and pharmacological challenges administered well after cessation of the stress paradigm.

274.2

We examined the effect of long-term treatment with antidepressant drugs on the tubulin polymerization in neonatal rats. To do this, we used the maximum concentration of acute and chronic stress (e.g., tone or variable) on tubulin assembly. The tubulin was prepared in buffer containing protein phosphate inhibitors, calmodulin A and sodium orthovanadate. The phosphorylation of microtubule was then monitored in vitro at 245 nm by a spectrophotometer. Treatment with desipramine, minaerin or citalopram for 7 days decreased the initial rate of tubulin assembly while a single injection of desipramine restored the rate. However, the tubulin reassembly without protein phosphate inhibitors nullified the inhibition of tubulin assembly following long-term desipramine treatment, suggesting that the inhibition of tubulin assembly is attributed to the phosphorylated microtubule-associated proteins (MAPs). MAP2 and tau factor. On the other hand, A single restraint stress increased the assembly rate, and the increase in the was recovered after chronic restraint stress for 7 days. In contrast, after chronic variable stress (cold-swimming, restraint, tail-pinch, shaking) for 7 days the increase in the rate was not recovered. It is thus proposed that the inhibition of tubulin assembly by the phosphorylation of MAPs may be an adaptive change to a novel stressor and this mechanism might be enhanced by long-term antidepressant treatment.

274.3
EFFECTS OF NEONATAL ISOLATION ON BEHAVIOR FOLLOWING AMPHETAMINE. C. Arner, R. P. Kohene and W. Sheenakar. Dept. of Psychiatry, UConn Health Center, Farmington, CT 06030, Dept. of Psychology, Trinity College, Hartford, CT 06106.

As part of a study on the ontogeny of behavioral and behavioral sensitivity we examined the proximal effects of neonatal isolation on locomotor activity and vocalizations following administration of amphetamine. One half of each litter was marked weighed and subjected to a daily one hour isolation from postnatal days (PND) 2-9. The other half of the litter was marked weighed and immediately returned to the nest and dam. Additional litters served as non-handled controls. Pups from these litters remained with their dams undisturbed through PND 9. On PND 10-19, pups were tested. Pups from each group were randomly divided into one of three challenge groups, saline 0.5 mg/kg amphetamine (APH) and 2.0 mg/kg APH. Animals were recorded for a five minute period beginning 15 minutes after drug administration. Ultrasonic vocalizations were recorded during this time with a bat detector. Females that were isolated and received 0.5 mg/kg APH showed higher levels of activity than the two control groups, significantly so in the course of patterned movements. These effects of isolation were not apparent in females tested with saline or 2.0 mg/kg APH. Males that were isolated and tested with 0.5 mg/kg APH, they showed lower activity scores than control males. Both doses of APH reduced vocalizations compared to saline. Among saline animals there was a clear effect of handling on the pattern of vocalizations over the five minutes period. There was a slight but insignificant trend for isolated animals to vocalize less than non-isolated, particularly with 0.5 mg/kg APH. These results suggest that neonatal isolation alters behavior following the administration of a low dose of amphetamine, at least when pups are tested 24 hour after the final isolation period. In addition, females seem to be affected to a greater degree than males, with regards to activity measures.

274.4

Stress can increase levels of cholesterol in plasma. Cholesterol is cleared from plasma primarily by high affinity cell-surface receptors. However, there is a secondary mechanism involving macrophages, a phagocytic immune cell. Thus, the effect of macrophage stimulation was examined as a potential mechanism for attenuating stress-induced increases in cholesterol. Rats (12 per group) were exposed to three sessions of inescapable tail-squeeze (100-160 mA shocks, one session per day for three successive days), or remained in their home cage. Half of each group were injected with zymosan (30 mg/kg, s.c.), a drug known to stimulate macrophage function in vivo. Animals received one zymosan injection for each of the three days prior to, as well as 15 min prior to each stress session. Control animals received vehicle injections. A week after a five-min period beginning 15 minutes after drug administration. Ultrasonic vocalizations were recorded during this time with a bat detector. Females that were isolated and received 0.5 mg/kg APH showed higher levels of activity than the two control groups, significantly so in the course of patterned movements. These effects of isolation were not apparent in females tested with saline or 2.0 mg/kg APH. Males that were isolated and tested with 0.5 mg/kg APH, they showed lower activity scores than control males. Both doses of APH reduced vocalizations compared to saline. Among saline animals there was a clear effect of handling on the pattern of vocalizations over the five minutes period. There was a slight but insignificant trend for isolated animals to vocalize less than non-isolated, particularly with 0.5 mg/kg APH. These results suggest that neonatal isolation alters behavior following the administration of a low dose of amphetamine, at least when pups are tested 24 hour after the final isolation period. In addition, females seem to be affected to a greater degree than males, with regards to activity measures.

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1769

174.5

Morris navigation performance was analyzed in adulthood (e.g., C57BL/6J) exhibiting hyper-corticosterone secretion in response to stressors, as well as particularly marked brain catecholamine alterations. Behaviorally, BALB/cByJ mice exhibited a wide array of behavioral disturbances after stressors that are more pronounced than in other strains. Interestingly, BALB/cByJ mice fail to acquire a spatial learning response in a Morris water-maze paradigm. In view of the markedly marked stress reactivity of the BALB/cByJ mouse, it was of interest to determine if the spatial learning deficit in this strain could be modified by neonatal manipulation, just as such a manipulation has been found to dampen stress responses in mature animals. We found that the performance deficit, which is not evident when visual inanamaze cues are present, is apparent as early as 30 days of age. Moreover, using a cross fostering paradigm it was determined that the deficits were not related to maternal factors. If pups were handled (i.e., separated from the Dam for brief periods over the course of the initial 21 days postpartum) the behavioral deficits were prevented. These data were related to the neurochemical and endocrine changes induced by stressors and the modification of such effects by neonatal handling.

174.6

Behavioral stress has effects on LTP and learning and memory. Autoradiographic studies of ligand binding to the AMPA receptor have also shown that the binding characteristics of the receptors are rapidly altered in the hippocampus and other brain structures following stress. While these studies reported a general increase in [35S]AMPA binding to striatal membranes, some reports (Bmax, or Kd), and type (high affinity or low affinity of receptor change have not been reported in detail. To evaluate the nature of the changes in AMPA receptors, we performed equilibrium saturation kinetic studies with [35S]AMPA on synaptic fractions (FD) prepared from both acutely stressed (60 tail shocks: 1 mA, 30-90 s interval) and control Long Evans rats. As previously reported, saturation kinetic data were better fitted to a two site vs a one site model, indicating the existence of a small population of high affinity sites (Kd = 20 nM) and a large number of low affinity sites (Kd = 600 nM). Stressed animals exhibited a significant increase in the maximal number of low affinity binding sites without significant changes in high affinity sites. As the low affinity receptors have been postulated to be synaptic receptors, whereas the high affinity receptors are enriched in nonsynaptic subcortical fractions, the results suggest that stress might alter some steps involved in receptor insertion or internalization. The results might also account for the impairment of LTP associated with stress, inasmuch as LTP shares the same cellular mechanisms of expression as via AMPA receptor activation. Supported by grants from NARS 1F32GM151021-01 BNR to JJK, NSF 9110378 to MB, and NSF BNS-8718300 & NIH (NIA) AG05142 to RTF.

174.7
BEHAVIORAL STRESS ENHANCES LTD IN RAT HIPPOCAMPUS. J. J. Kim1, M. R. Foy2, and R. F. Thompson1.
1Neurosciences Program, Univ. of Southern Calif, Los Angeles, CA 90089-2520 and 2Psychology Dept., Loyola Marymount Univ., Los Angeles, CA 90045-2669.

Uncontrollable and unpredictable stress is known to impair certain learning tasks, and also to impair hippocampal long-term potentiation (LTP). In the present study, we examined the effect of stress on another form of hippocampal synaptic plasticity, long-term depression (LTD). Hippocampal slices were prepared from adult Long Evans rats immediately following exposure to behavioral stress (60 tailshocks: 1 mA x 1-3, 30-90 s apart; animals immobilized). Field EPSP recordings (amplitude and slope) from dentrites in area CA1 following Schaffer/commisural stimulation (900 pulses at 1 Hz) indicate that LTD was significantly greater in stressed animals than that recorded from control animals. These results argue that an additional form of hippocampal plasticity can be modified by behavioral stress.

Supported by grants from NARS 1F32GM151021-01 BNR to JJK, LMU to MRF, and NSF BNS-8718300 & NIH (NIA) AG05142 to RTF.

174.8
RECURRENT BRAIN POLYAMINE RESPONSE AFTER REPEATED STRESS AND ITS ENHANCEMENT AFTER AN ADDITIONAL DELAYED STRESS EPISODE. V.R. Gilad5 and G.M. Gilad. Faculty of Med., Technion-Israel Inst. of Technology, Haifa, Israel.

Rapid changes in brain polyamine (PA) metabolism, termed the PA response, are generally induced with a magnitude proportional to the stressor intensity. The effects of repeated stress episodes on the PA response are unknown. Therefore, we examined effects of repeated restraint, a relatively mild stressor, on diacetylcarnitine decarboxylase (DCC) and 8-adenosylmethionine decarboxylase (SAM-DCC), two key PA synthesizing enzymes. Rats were subjected to 2 h restraint once daily for 5 days, and enzyme activities, assayed in the hippocampus 6 h after the beginning of each stress episode. We found that DCC activity was increased repeatedly after each stress episode to about 130% of control. In contrast, SAM-DCC activity was reduced after the first episode (86% of control), but remained unchanged thereafter. Additional stress application, after a 24 h break in the last 5th episode, resulted in a much larger DCC increase (180% of control), without a change in SAM-DCC activity. We conclude that: 1) even mild stress can induce a characteristic PA response in the brain; 2) the OCD response is induced after each episode of stress, and 3) additional stress episode applied days after cessation of the initial stressful stimuli, results in a much greater increase in OCD activity. The study implicates an overactive PA response in the (mal)adaptive response to stressful events.

174.9
CHANGES IN BOMBESIN (BN) LEVELS AND ITS RECEPTORS IN THE RAT BRAIN FOLLOWING ACUTE IMMUNOZIMMERSION STRESS. P. Kent1, Z. Meraj2, and H. Anisman1.
1Psych & 2Pharmacol, Univ of Ottawa, 2Carleton University, Ottawa, Ontario, Canada.

The neurochemical mechanisms underlying the coincident activation of the pituitary-adrenocortical and the sympathetic nervous system in response to stress remain unclear. Central injection of the neuropeptide bombesin (BN), potently releases epinephrine from the adrenal medulla and adrenoreceptors located in the pituitary, and elicits behaviors typically associated with increased emotionality and arousal. The objective of the current study was to determine whether stress is associated with changes in the endogenous levels of BN-like peptides and/or their receptors. Male Sprague-Dawley rats were subjected to acute immobilization stress for 0 (control), 30 min or 120 min. Plasma ACTH levels increased in response to stress, peaking at 30 min. BN-like Immunoreactivity increased significantly at the hypothalamus and medulla, within 30 min, and declined to control levels by 120 min. The peptide levels in several other regions, including the hippocampus, striatum, midbrain, pituitary, and pons, failed to change significantly. Autoradiographic analysis of BN/GRP receptor densities for 8 regions examined are listed in ascending order: hippocampus, nucleus tractus solitarius (NTS), paraventricular (PVN), arcuate (Arc) and paraventricular hypothalamic nuclei, paraventricular thalamic nucleus, central amygdala and nucleus accumbens. Significant increases in binding were found after 30 and 120 min of stress in the NTS and after 120 min in the PVN and Arc. These data indicate that BN-like peptides may serve to mediate and/or integrate responses to stress.

174.10
THE PROTECTIVE EFFECTS OF STRESS CONTROL MAY BE MEDITATED BY INCREASED BRAIN LEVELS OF BENDAZEPINE RECEPTOR ANTIGENS. R.C. Drugan, A.S. Basile, J.H. Ha, and R.J. Ferland. Department of Psychology, Brown University, Providence, RI 02912 and Laboratory of Neurosciences, NINDS, NIH, Bethesda, MD 20892.

Control over stress protects against many of the deleterious effects of stress exposure, but the endogenous mediators responsible for these prophylactic effects are not known. In vitro radioligand binding and neurochemical analyses, we demonstrate that exposure to escapable stress results in brain and behavior changes reminiscent of benzodiazepine administration. The stress control group shows significant protection against picrotoxin-induced seizures, reductions in [3H]5-bromo-5-chloro-1,3-dihydrobenzodiazepine binding at or near the chloride channel and a 3-fold increase of benzodiazepine-like agonists in brain comparison to both yoked-inescapable shock and non-shock controls. These observations suggest that active coping behavior leads to the release of endogenous benzodiazepine-like agonists that protect the organism from stress pathology. Research supported by NIH grant MH 45475 to RD.

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724.11
ALCOHOL AVAILABILITY AND AGGRESSION LEVEL AFFECT BEHAVIOR IN THE OPEN FIELD AND PLUS MAZE, AND 5-HT RECEPTORS IN TRIAD-HOUSED RATS. L.A. Podolec*, X. Huang, S. Lason, C. Quinn, D. Benjamin, Center of Alcohol Studies, Rutgers University, Piscataway, NJ 08855.

Individually- and triad-housed male Long Evans rats were tested in the open field and elevated plus maze. Subjects were 18 triads and 14 single caged rats, half of which received 6% solution of ethanol (ET) or water. Dominance status was determined by monitoring behavior during the initial 30 minutes of colony formation. Initially, the number of attacks by the Alpha (α) on the Beta (β) and Gamma (γ) was observed daily to classify triads by aggression level. Body weight loss 1 day after group housing was similar for β and γ but minimal for α, and singles gained. Lean weight gain was observed in high aggression (HT) rats in middle aggression (MT) or low aggression triads (LT).

Weight gain of α was not affected by aggression level. Only the β were affected by ET availability, with lower weight gain overall. Headpokes were affected by level of aggression; in LT headpokes were less frequent, and of shorter total duration, but in MT, β showed the lowest duration and frequency. In the LT, ET increased headpoking duration and frequency. In triads with ET available, α had significantly lower headpoking frequency. In the MT, ET decreased grooming, but had no effect in LT. In triads with ET available, the MT showed significantly less grooming than MT or LT. In the β, availability of ET increased central tone significantly. The availability of ET produced unidirectional effects in singly housed, but not in triad-housed rats. 5-HT1A receptor binding was significantly affected by aggression level; whereas ET showed rank-related increases above control levels in β and γ, LT showed rank-related decreases in β and γ. Effects on 5-HT1A binding were also observed. These results demonstrate that aggression levels vary between ET availability and triad exemplars effects on 5-HT1A receptor binding and behavior (Supported by NIAAA 05396).

725.1
TEMPORAL REGULATION OF PREPROENKEPHALIN-A (PPE-A) mRNA EXPRESSION BY ESTROGEN IN THE POSTERIOR DORSAL MEDIAL AMYGDALE (MeApd) OF THE FEMALE RAT. C. B. Eckerson*, C. A. Printz and P. E. Morecraft, Dept. Anatomy and Cell Biology, Laboratory of Neuroendocrinology, UCLA School of Medicine, Los Angeles, CA 90024.

Gonadal steroids modulate reproductive behavior through their effects on intercellular signaling in the limbic-hypothalamic neural circuit. Within this circuit, the up-regulation of PPE-A mRNA (encoding pre-pro-enkephalin) by estrogen, in the ventromedial nucleus of the hypothalamus, has been correlated with reproductive behavior in the female rat. The MeApd also is an integral part of the limbic-hypothalamic neural circuit that plays an important role in opioid regulation of female reproductive behavior. To examine the temporal effects of acute estrogen exposure on PPE-A mRNA levels in the MeApd, adult ovariectomized Long-Evans rats were injected with 50 μg estradiol benzoate (EB); perfused at one of nine successive time points from 0 to 72 hours after injection and used for quantitative in situ hybridization histochemistry of PPE-A mRNA. PPE-A mRNA was localized to neurons within the MeApd using an α2,β-labeled, single-stranded, RNA probe complementary to the entire coding sequence of the PPE-A mRNA (gift from Drs. Yoshikawa and Kato, NHI, Bethesda, MD). EB treatment induced a biphasic increase in the number of PPE-A mRNA-expressing cells in the MeApd with maximal levels occurring at 1 and 24 hours post treatment. These peaks were interrupted by a dramatic decrease in the number of PPE-A mRNA expressing cells at 4 hours after EB injection. By 72 hours post-EB, PPE-A expressing cell numbers returned to basal levels. The biphasic increase in PPE-A mRNA expressing cell numbers with acute estrogen treatment supports the idea that there are two temporal and perhaps mechanistic regulatory events for PPE-A transcription within the MeApd. Supported by NS 21120.

725.2
PROGESTERONE ENHANCES AN ESTRADIOL-INDUCED INCREASE IN FOS EXPRESSION BY HYBRIDIZATION IN THE POSTERIOR DORSAL MEDIAL AMYGDALE (MeApd) OF THE FEMALE RAT. A.P. Auger and J.D. Blaustein*, Neurosci. & Behav. Program, Univ. of Massachusetts, Amherst, MA 01003.

Female rats. The temporal control of reproductive behavior depends on the sequential presence of estradiol followed by progesterone. Although treatment with high doses of estradiol has been shown to increase local immunostaining for Fos protein, an immediate early gene product that is expressed upon cellular activation, another report conflicts with this finding. However, the previous reports agree that subsequent treatment with progesterone has no apparent effect on Fos expression. In order to resolve this discrepancy and investigate possible effects of progesterone, we used Fos immunocytochemistry combined with computer-aided image analysis. In experiment one, we found that treatment with 5 μg of estradiol increased Fos immunoreactivity (Fos-IR) within the medial preoptic area and the dorsal medial hypothalamus. Subsequent treatment with 500 μg of progesterone one hour before perfusion increased the intensity of the immunostaining within the medial preoptic area and the dorsal medial hypothalamus, although it had no significant effect on Fos-IR cell number. In experiment two, a lower concentration of Fos antisera was used in order to diminish the immunostaining sensitivity to a level in which no increase of Fos-IR cell number was observed after treatment with estradiol. Under these immunocytochemical conditions, subsequent treatment with progesterone increased the number of Fos-IR cells in the medial preoptic area, the dorsal medial hypothalamus and the steroid receptor-rich area lateral to the ventromedial hypothalamus. Thus, treatment with behaviorally-effective doses of both estradiol and progesterone induces Fos expression in localized regions of female rat brain. (supported by NS 19327 from NIH and RSDA MH 00685 from NIMH)

725.3

Progesterone (P) effects on extracellular (EC) 5HT were monitored in the ventromedial hypothalamus (VMH), midbrain central grey (MCG) or medial preoptic area (mPOA) using microdialysis. Rats primed with estradiol benzoate (5 μg) were anesthetized with chloral hydrate (400 mg/kg, i.p.). Samples were collected at 20 min intervals. Once a stable 5HT baseline was obtained, rats were injected (s.c.) with 0.5 mg xylazine (V). In the mPOA, EC 5HT levels were not affected by P. Significant decreases in EC 5HT levels were present in the MCG and VMH 40 and 60 min after P, respectively. In the VMH, EC 5HT decreased to 57 ± 5.9 % of pretreatment values 100 min after P. 5HT levels in the MCG decreased to 56 ± 5.1 % of pretreatment values 80 min after P. The decreases in EC 5HT in the VMH and MCG persisted during the remainder of the sampling period. 5HT baseline levels were stable in the respective V-treated groups. These results demonstrated thatinfusions decreases 5HT release in the MCG and VMH occur in vivo and support previous studies which suggest that decreases in 5HT activity in the VMH and MCG contribute to the facilitation of female receptivity. (Supported by NIH 3909451)

725.4
SUBCELLULAR LOCALIZATION AND KINETIC PROPERTIES OF AROMATASE IN THE RAT BRAIN. C.E. Rosselli*, Department of Physiology, Oregon Health Sciences University, Portland, OR 97201.

The conversion of testosterone (T) to estradiol is catalyzed by cytochrome P450 aromatase. In situ aromatization is required for the full expression of the effects of T in the brain. This study examined the subcellular distribution and reaction kinetics of aromatase in the adult rat brain. Preoptic area (POA), hypothalamus (HYP) and amygdala (AMYG) were homogenized in isotonic sucrose buffered with potassium phosphate. Homogenates were fractionated by a sequential series of centrifugations to obtain: 800 g pellet (nuclei); 11,000 x g pellet (mitochondria and synaptosomes); 100,000 x g pellet (microsomes); and 100,000 x g supernatant (cytosol). Aromatase activity (AA) was measured using a previously validated 1H2O assay. Marker enzymes were measured to identify organelles in the different subcellular fractions. We found that AA in all 3 tissues was enriched 8-14 fold in microsomes, but not in other cell subfractions. The addition of either a NADPH-generating system or 1 mM NADP+ to the reaction mixture stimulated AA in all subfractions, whereas NADH was only minimally effective. Affinity constants were equivalent in all subfractions (~ 10 μM) suggesting that only one form of the enzyme is present in the rat brain. One week after castration, AA was significantly reduced in all cell subfractions of POA and in the homogenate and microsomes of HYP. Castration did not significantly alter AA in any subfraction of AMYG. To more critically evaluate subcellular localization, AA was measured in purified synaptosomes. AA was not enriched in these preparations suggesting that aromatase is not substantially associated with nerve terminals in rat brain.
T25.5

**HYPOTHALAMIC OPIOID RECEPTORS IN MALE JAPANESE TELEOST FISH, HAPLOCHROMIS BURTONI.**

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Galanim, a 29 amino acid peptide present in both gut and brain, has been called a hypothalamic-hypophysiotropic hormone because it can effect release of pituitary hormones such as growth hormone, prolactin, and gonadotropins. Galanim and its mRNA can be co-localized in gonadotropin-releasing hormone (GnRH) cells in the hypophyseal/pretorpic area (POA); although it is difficult to visualize the role of galanim in regulating GnRH release in the teleost fish, H. burtoni. Males of this species exhibit size changes in the POA of GnRH cell population that are correlated with changes in social status and reproductive maturity. These changes in cell size are plastic; that is, up-grading or down-grading the social status of an adult H. burtoni male results in a corresponding increase or decrease in the size of the GnRH cells in the POA. We addressed three questions: 1) Is there a galanim-like immunoreactivity (GAL-LI) present in the POA of H. burtoni males? 2) Do these GAL-LI cells project to the pituitary? and 3) Do these GAL-LI exhibit a correlation between cell size and social/adaptive productivity similar to that which has been reported for GnRH cells in the POA? H. burtoni males were observed behaviorally for 4 weeks to establish social status, then sacrificed, and cryostat sections of the POA were stained for GAL-LI. We observed that 1) GAL-LI cells were present in the POA of all males, 2) the majority of these GAL-LI cells did not contain fast DI suggesting that only a very small minority of these cells may project to the pituitary, and 3) the GAL-LI cells showed a tendency towards a correlation between social status and cell size with socially dominant males having larger GAL-LI cells although the size differential was small.

Supported by NIH HD 23799 to RDP and NSERC post-doctoral scholarship to TB.

T25.6

**EVOLUTION OF THE GnRH FAMILY OF PEPTIDES INFERRED FROM cDNA SEQUENCES.**

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The decapeptide GnRH is an important regulator of vertebrate reproductive function and has been highly conserved during the course of vertebrate evolution. Most, and perhaps all, vertebrates express at least two forms of GnRH. Chicken-I GnRH (cGnRH-I) is the most ubiquitous form, and has been identified in every major vertebrate taxon. Previously it was thought that eutherian mammals do not express cGnRH-II but the gene encoding this form has now been cloned and sequenced in members of this taxon as well. While it has potent gonadotropin releasing action in vitro, cGnRH-II neurons are generally confined to midbrain regions, and with the possible exception of elasmobranchs, are not thought to function in gonadotropin release in vivo. In this study we used a variety of evolutionary distance measuring and phylogenetic techniques to analyze amino acid and cDNA sequences, both to explore the evolutionary history of this family of peptides and their functional divergence across vertebrates. Our results suggest that there are two distinct GnRH lineages, which we call GnRH-I and GnRH-II, which resulted from a single gene duplication event early in the course of vertebrate evolution. The GnRH-I lineage includes mammalian GnRH (mGnRH), chicken-I GnRH (cGnRH-I) and salmon GnRH (sGnRH) and all other forms of placental origin. The GnRH-II lineage includes (cGnRH-II and other forms expressed in the midbrain. All forms of GnRH exhibit, at both the cDNA and amino acid levels, a high degree of sequence identity in the GnRH region, somewhat less loss so in the signal sequence, and markedly less so in the associated peptide coding region. All GnRH-I forms have a single associated peptide (GAP), while GnRH-II forms have two associated peptides. The low degree of conservation of these associated peptides implies that whatever function they serve, if any, requires little in the way of sequence specificity.

T25.7

**THE POSSIBLE ROLE OF NPY Y1 RECEPTORS IN THE CONTROL OF LORDOSIS.**

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Previously we have shown that NPY plays a facilitatory role in the control of lordosis. These studies examined if NPY acts at Y1 receptors to affect lordosis. Adult guinea pigs were ovariectomized and implanted with a cannula into the lateral ventricle. In Exp 1, females were injected with 20ng Estradiol Benzoate (EB) followed 40h later by .5mg Progesterone (P). Females which displayed lordosis after P were injected ICV with either 0.5 or 5 ug of the NPY 1 agonist 1e3u1, pro34 NPY, or were used as controls. The 5 ug dose of the Y1 agonist significantly increased lordosis responding. In Exp 2, ovariectomized females were injected with 20ug EB and tested for lordosis 40h later. They were then injected ICV with NPY 1 agonist (5ug) or were used as controls. The Y1 agonist did not facilitate lordosis in these females. These data suggest that 1) NPY acts at Y1 receptors to facilitate lordosis and 2) NPY may affect some progesterone mediated component of lordosis since only EB+P but not EB induced lordosis was facilitated.

T25.8

**AQUEOUS EXTRACT ACTION OF RUTA CHALEPENSIS ON SPERM MOTILITY.**


Muccitelli and Ferguson (1) point out flaws in the experimental design of a recent study by Kupitz and Atlas (2), where the experimental condition do not guarantee that the applied concentration of extract was present at the membrane surface of the mature egg, and that a more likely target for the compounds is the sperm if any of the applied compounds interfered with sperm activation or motility. This prompted us to tested aqueous extract of Ruta chalepensis on rat sperm motility, as it has been suggested that Ruta chalepensis extract has an anticonceptive action, and it is used in traditional medicine as vaginal cream before sexual intercourse. Exposing sperm recently obtained from rat testis to Ruta chalepensis extract, we found under light microscope: reduction in motility, decomposition of tail motility, until complete hamobilization of spermatozoa without determining the involvement of ion channels. This Ruta chalepensis effect may play a role in its postulated anticonceptive action.1. Science 263:198, 1994; 2. Science 261:484, 1993.

Glutamate receptors may play an important role in the pathophysiology of schizophrenia and the motor side effects of chronic neuroleptic drug treatment. We examined changes in the binding of three glutamate receptor subtypes using [3H]-MK801 (NM3DA receptor antagonist), [3H]-CNQX (AMPA-R antagonist) and [3H]-Kainic acid (kainate-R antagonist) in vitro receptor autoradiography following one month's treatment of rats with SCH23390 (0.5 mg/kg/day), clozapine (CLZ) (25 mg/kg/day) and haloperidol (HAL) (1.5 mg/kg/day), or raclopride (RAC) (10 mg/kg/day). Chronic SCH treatment elevated [3H]-MK801 binding in the hippocampal formation with significant increases in CA3 and dentate gyrus. This result suggests a specific role for dopamine D2 receptors in the regulation of hippocampal NMDA receptor function. In contrast, haloc or Rac treatment did not alter [3H]-MK801 binding in any of the brain regions analyzed. However, chronic CLZ treatment caused a significant decrease in [3H]-MK801 binding in caudate putamen (medial: -34%, lateral: -22%) without affecting the hippocampal formation, nucleus accumbens or the cortex. This might explain why it produces minimal extrapyramidal side effects. Results for AMPA and kainate receptors will also be reported. Supported by MH44211 and Sigma Xi grant (F.I.T.)

726.2  REGULATION OF DOPAMINE RECEPTOR BINDING FOLLOWING CHRONIC NEUROLEPTIC TREATMENT. F.J. Tarazi*, W.J. Florin and Jan Creese. Center for Molecular and Behavioral Neuroscience, Rutgers University, New Jersey, NJ 07848.

We examined changes in dopamine receptor binding in various brain regions following one month's treatment of rats with SCH23390 (0.5 mg/kg/day), haloperidol (HAL) (1.5 mg/kg/day), raclopride (RAC) (10 mg/kg/day) or clozapine (CLZ) (25 mg/kg/day) using in vitro receptor autoradiography. Chronic SCH treatment significantly increased [3H]-SCH23390 binding in nucleus accumbens (NA) [+55%] and caudate putamen (CP) [+28%] and in NA [+89% and 45%]. Rac treatment also elevated [3H]-YM-091512-I and [3H]-spiperone binding in CP (+28% and +25%). [3H]-Raclopride binding was only increased in the lateral CP [+17%] of HAL-treated rats. Since [3H]-YM-091512-I and [3H]-spiperone have similar affinities for D2, D3 and D4 receptors but the raclopride has low affinity for D4 receptors, these results suggest that D2 receptors are majorly upregulated by the HAL treatment. Thus, [3H]-YM-091512-I and [3H]-spiperone binding in the presence of a D2 and D4 receptor saturating concentration of raclopride (300 nM) to quantitatively selectively putative D2 receptors was significantly increased in both the CP and NA (+70% of HAL-treated rats). Interestingly, the binding of both in radioligands in the presence of 300 nM raclopride was significantly increased in CP (+26%) but not in NA of CLZ-treated rats. Supported by MH44211 and Sigma Xi grant (F.I.T.)

726.3  AKATHISIA-LIKE RESPONSE TO HALOPERIDOL IN RATS TRAINED TO PERFORM A SUSTAINED ATTENTION TASK. B.J. Brockel* and B.C. Fowler. Univ. of Mississippi Medical Center, MS 38677.

Rats were trained to remain immobile with their heads in observation tunnels where a brief (0.12 s) visual stimulus were presented. The rats learned to react to 1 of 3 visual stimuli by executing a nose-poke response within 2 s of stimulus presentation. Exits from the tunnel were penalized by a 7.5-s time-out, a contingency that made the rats refrain from locomotion for most of the 900-s session. Forty animals were equally divided into 5 separate groups with each group receiving its own dose (0.0 to 0.12 mg/kg) of haloperidol (HAL) daily for 23 days. HAL decreased reinforcers and dose-dependently increased proportion of errors of omission, suggesting that HAL not only reduces responding but also produces attentional deficits. Number of head exits from the observation tunnel was dose-dependently increased by Hal. This effect showed gradual tolerance across the chronic dosing. Head exits in this paradigm may serve as an animal model of akathisia. Supported by MH43429.

726.4  HALOPERIDOL-INDUCED MONOAMINE DEPLETION: TYROSINE HYDROXYLASE-NEGATIVE SUBSETS OF DOPAMINE NEURONS ARE AFFECTED IN GASTROPOD CNS. D. Bakkarinen*, E. Vaneckova, I. Mihalikova, M. Baker, B. Croft*.


Long-term treatment with haloperidol (HAL) was shown to result in depletion of specific subsets of putative dopamine (DA) neurons (Neuroreport 1994, 5:67). With the aim of further investigating what appears to be a novel action of HAL, the present study was performed on adult and juvenile specimens of the pond snail Lymnaea stagnalis kept for up to 40 days in 0.5-2.0 µM HAL solutions. HPLC-EC analysis of the CNS revealed a concentration-dependent DA depletion which reached 20-50% over the first 4-8 days and thereafter remained stable. A comparable DA depletion was observed in the leaf meal Achatina fulica as a result of daily HAL injection, 1 µg/kg for 4 days). In cats, a transient, dose-independent, serotonin depletion was also observed which, by day 12, reached to 60% with follow-up confirmed (for 0.5 µM HAL) or partial recovery by day 20 of treatment. Glycine-mediated-induced neurodegeneration was found undetectable in some, but not all, identifiable clusters of DA neurons in the CNS of animals treated with HAL for 3-4 days or more. A peculiar feature of HAL-sensitive DA neurons was that they were non-reactive to antibodies raised against tyrosine hydroxylase, whereas HAL-resistant DA neurons showed such reaction. Thus, the previous and present reports suggest that the depleting action of low doses of chronic HAL observed in specific subsets of DA neurons may be a phylogenetically conserved characteristic which, if generalized to vertebrates, may account for therapeutic effects of this and related drugs.


726.5  NITRIC OXIDE SYNTHESIS INHIBITION ATTENUATES HALOPERIDOL-INDUCED SUPERSENSITIVITY. C.M. Pudinsak*, M.A. Bozarth. Department of Psychology, University at Buffalo, Buffalo, NY 14260-4110.

Previous work has shown that nitric oxide is involved in sensitization to the locomotor-stimulating effect of repeated cocaine injections (Pudinsak & Bozarth, Life Sci. 53: 1517-1524, 1993.). Increased responsiveness to stimulants can also be produced by chronic dopamine-D2 receptor blockade. This study examined the effectiveness of an oxime synthesis inhibitor in blocking the supersensitization following chronic neuroleptic treatment (i.e., dopamine-receptor blockade). No-nitro-L-arginine-Na (L-NAME) was used to inhibit nitric oxide synthesis, while haloperidol was used to produce supersensitivity by chronically blocking dopamine receptors. Male, Long-Evans rats were tested in locomotor activity chambers for 30 min following an injection of normal saline (1 ml/kg, i.p.). On the next day, they were retested following an injection of cocaine hydrochloride (10 mg/kg, i.p.). After that they were treated daily with haloperidol (0.2 mg/kg, i.p.) for 14 days. Half of the rats were pre-treated with cocaine (0.5 mg/kg, i.p.) and haloperidol (HAL-NAMe, 30 mg/kg, i.p.) 30 min before their haloperidol injections. Seventy-two hours after their last injection, rats were injected with cocaine (10 mg/kg, i.p.) and were tested for 30 min in locomotor activity boxes. Rats treated with saline plus haloperidol showed greater stimulation of locomotor activity than their first cocaine test, while rats treated with L-NAME plus haloperidol showed little change in activity. This finding suggests two possibilities: the activation of nitric oxide synthesis antagonized the haloperidol-induced supersensitivity to cocaine. Similar mechanisms may be involved in neuroleptic-induced supersensitivity and stimulant-induced sensitization.

(Supported by DA00285 from the National Institute on Drug Abuse.)


Antipsychotic drugs are known to cause a variety of neuropsychiatric side effects including the precipitation of seizures. However, as yet there is no animal model which is predictive of seizure liability. In the present study, several clinically used antipsychotic drugs were evaluated for their ability to affect the onset of seizures induced by infusion of the chemical convulsant PTZ in rats. In this species, PTZ-induced seizures were not only electrocorticographically detectable in some, but not all, identifiable clusters of DA neurons in the CNS of animals treated with HAL for 3-4 days or more. A peculiar feature of HAL-sensitive DA neurons was that they were non-reactive to antibodies raised against tyrosine hydroxylase, whereas HAL-resistant DA neurons showed such reaction. Thus, the previous and present reports suggest that the depleting action of low doses of chronic HAL observed in specific subsets of DA neurons may be a phylogenetically conserved characteristic which, if generalized to vertebrates, may account for therapeutic effects of this and related drugs.

876.7

Social isolation of postweanling rats results in a syndrome of behaviours and a pattern of biochemical alterations that are at least in part, indicative of enhanced dopaminergic activity. Given the postulated role of dopaminergic overactivity in schizophrenia, and the need to develop new, non-pharmacological therapeutic models, the present study began investigating the behavioral effects of social isolation in rats, and interactions with antipsychotics. Male Wistar rats were housed either singly ("isolated" condition) or in groups of 4 ("condition") beginning at 25 days of age. When tested 5 weeks later, isolated rats placed in a novel test environment showed a significant locomotor hyperactivity relative to grouped rats. This hyperactivity was particularly notable during the first 10 min of testing when initial exploratory levels of activity are high. Isolated rats also tended to show decreased pupped prepulse inhibition of acoustic startle using either auditory prepulse (weak white noise burst) or a visual prepulse (light flash). Dose-response studies indicate that the locomotor hyperactivity can be reduced by the DA antagonist haloperidol at doses that did not significantly reduce baseline levels of activity. Preliminary findings of increased dopamine concentrations in the striatum and n. accumbens are also consistent with enhanced dopaminergic activity in the isolates. These data support the conclusion that the locomotor hyperactivity produced by social isolation is attributable, at least in part, to dopaminergic hyperactivity.

876.9

In addition to dopamine receptors, both a-adrenoreceptors and adrenoreceptors are implicated in the pathogenesis and treatment of schizophrenia. Riociguat (RIS) is of interest in view of its high affinity at 5-HT2A/2C receptors vs. D2 receptors (Ki = 9.28 ± 0.8 vs. 83) as compared to haloperidol (7.99 ± 0.8 vs. 87). Furthermore, it was recently proposed that m/mic (male) rats are less susceptible to the apomorphine-induced stereotypies than female rats. We used the S 17828 and S 14956, novel benzimidazoles, to investigate the potential of RIS in treating apomorphine-induced hyperactivity. In a second study, we show that the novel benzimidazoles, S 17828 and S 14956, also manifest high affinity at 5-HT-2A/2C receptors vs. D2 receptors; pKi were 8.38/7.28 vs. 3.73 for 5-HT, and 8.5 vs. 3.72 for D2. Interestingly, the S 17828 and S 14956, in the same order, show low affinity at D1 sites as compared to RIS (8.9). Ex vivo, in (male) rats, S 17828 and S 14956 mimicked the in vivo effect of S 17828. Since these compounds may be useful in the treatment of schizophrenia and other neuropsychiatric disorders, further studies are warranted.

876.10

Disruption of LI, that is, the inability to ignore information that is irrelevant to the task at hand, has been proposed as a model for the attentional-cognitive deficits of schizophrenia. In the present study, we characterize this model by use of a three-step paradigm. After establishment of a stable baseline of behavioral indices (e.g., rate of errors), rats were divided into two groups: one first pre-exposed (PE) or not pre-exposed (NPE) to a series of tones. One day later, during a conditioning session, they were exposed to two tone-shock pairings and a 24 h. test, on the day following, rats were allowed to react as in the test. The time to completelicks 90-100 (s) and licks 100-110 (s) were measured and the suppression ratio (SR) was defined as 1-(s90/1) / (s100/1). It was found that genotype (200 vs. 300 g), pretreatment (combinations of either 600 or 1000 licks within 10 min), duration of pre-exposure (40 tones/40 min vs. 40 tones/15 min) and the duration of the interval between pre-exposure and testing (24 h. delay between sessions but not failed to disrupt LI in rats that received conditioning were performed on the same day. Under the former conditions, haloperidol, 2 x 0.1 and 2 x 0.16 mg/kg, i.p., evoked LI in rats pre-exposed to 10 tones. It is concluded that, in the LI-CER model, the intensity of the unconditioned stimulus is decisive for the induction of LI shift and that, based on the results obtained with d-j-amphetamine and haloperidol, this paradigm is an appropriate model for the characterization of antipsychotic drugs.

876.11

The antipsychotic sertindole (1,2-D,4,6(1,4)-chloro-1-(4-fluorophenyl)-indole-3-thiodiazole 2); is a poliolide, with high affinity for strionetion-5-HT1D (K(i) = 49.0 nM), 5-HT7, 5-HT4, 5-HT2A, and dopamine D2 receptors (K(i) = 4.1 nM) and receptors for a-adrenoreceptors (K(i) = 4.9 nM). In vivo studies have a long history in the rat (inhibition of amphetamine-induced head twitches; ED50 = 0.039 µg/kg, 24 hours after p.o. administration). Despite high affinity in vitro for dopamine receptors sertindole exerts no catalepsy, no EPS in the rat with 5-HT antagonists and no EPS in the rat with D1 antagonists and no EPS in the rat after 2 weeks of treatment. The receptor binding profiles will be presented and discussed in relation to the effects on A10/A9 dopamine neurons after chronic administration with the sertindole-induced inhibition of A10 dopamine neurons was found for some compounds. Results for the antipsychotics haloperidol, clozapine, and remoxpine will be included for comparison.

876.12

SM-13496 is a newly discovered antipsychotic that preferentially acts on dopamine D2 and 5-HT receptors. SM-13496 showed high to moderate affinities for D2 (K(i) = 1.44 nM) and 5-HT (K(i) = 26.8 nM) but had negligible affinities for D1, 5-HT, 5-HT1a, 5-b, muscarinic, GABA, benzodiazepine, a1, or glutamate receptors. Oral administration of SM-13496 blocked dopaminergic behaviors (e.g., methamphetamine-induced hyperactivity in rats and apomorphine-induced climbing behavior in mice) and selectively suppressed the conditioned avoidance response in rats (ED50 = 1.7 ± 0.9 mg/kg, i.p.). SM-13496 also inhibited 5-HT2 receptor-mediated behaviors (e.g., tryptamine-induced clonic seizure and p-chloroamphetamine-induced hyperactivity in mice) with negligible antipsychotic activity in the Vogel's conflict test (MED = 10 mg/kg).

Despite its potent D2 blocking activities, SM-13496 showed only negligible actions in inducing extrapyramidal side effects (i.e., catalepsy and bradykinesia, ED50 >1000 µg/kg, potentiation of anesthesia (ED50 >1000 µg/kg), muscle relaxation (ED50 >1000 µg/kg) and inhibition or potentiation of convolution (ED50 >1000 µg/kg). In conclusion, SM-13496 is a novel antipsychotic with minimal extrapyramidal and CNS depressive side effects.
726.13

5-HT1A RECEPTOR BLOCKADE BY SM-9018, A NOVEL 5-HT1A AND D2 ANTAGONIST, COMPETITIVELY BLOCKS ITS INTRACEREBRAL EFFECTS ON STRIATAL Dopamine D2 Receptors 

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SM-9018 is a potent atypical antipsychotic that has potent 5-HT1A and D2 blocking activities. To elucidate the role of its 5-HT1A blocking activity in the striatum, we studied the effects of SM-9018 (5-HT1A/D2 antagonist) on haloperidol (D2 antagonist) and 5-HT1A antagonist on the induction of extrapyramidal side effects (i.e., bradykinesia), ex vivo c-fos mRNA expression in the striatum and in vitro acetylcholine (ACh) release from striatal slices. Antipsychotics (SM-9018, haloperidol) dose-dependently induced bradykinesia in the pole-depending behavior of mice with relative potencies consistent with their own in vitro acetylcholine release. Induction of bradykinesia was about 70 times weaker than haloperidol in inducing bradykinesia and had a 13 times higher therapeutic index (ant-apoliprotein/b Tradesomen) ratio. 5-HT1A antagonists (e.g., raloxifene) had no effects by themselves, but significantly reduced the haloperidol-induced bradykinesia. Oral administration of haloperidol markedly enhanced c-fos mRNA expression in the rat striatum (about 7 fold at 30 mg/kg) whereas SM-9018 showed only a slight increase (about 1.8 fold) at doses of up to 30 mg/kg. 5-HT1A antagonists failed to affect c-fos mRNA expression by themselves, but markedly attenuated the haloperidol-induced c-fos expression. Finally, SM-9018 was about 10 times weaker than haloperidol in enhancing the in vitro AChrelease upon 3Hz electrical stimulation while it was as potent as haloperidol in reducing D2 receptors and in antagonizing the inhibitory effects of exogenous quinpirole (a D2 agonist) on [3H]ACh release. These results suggest that the 5-HT1A blocking activity of SM-9018 counteracts its antinoceptive actions at the striatal D2 receptors, which seem to contribute to its low incidence of extrapyramidal side effects.

726.15


Intrinsic activity may be an essential determinant of the clinical antipsychotic profile of partial dopamine (DA) 2 agonists. In order to determine the utility of preclinical tests for estimating intrinsic activity we measured the effects of a series of partial DA agonists in models commonly used to characterize dopamine DA2 agonists in vivo and compared the results to the maximal inhibition of forskolin-stimulated cyclic AMP accumulation in GH4C1 cells expressing the human D2 receptor in vitro (cAMP). In vivo tests included subcutaous nigra dopamine neuron firing (DNR), gamma-butyrolactone-stimulated brain dopamine synthesis (GBL) and intracerebral microdialysis (IDM) in rats. Drugs were tested at 1 μM in cAMP, a level verified as supramaximal for selected agonists. Dose-effect curves were determined for DNR (i.v.) and high single doses were used for GBL (i.p.).

Relatively intrinsic activity in cAMP was: apomorphine > B-HT920 = EMS38352 > CI-1007 > terguride > (-)-3-PPP > SDZ 912 > haloperidol. Maximal effects in DNR and GBL revealed a similar order of intrinsic activity. However, maximal effects in DNR and GBL suggested: apomorphine = B-HT920 and CI-1007 > (-)-3-PPP. While apomorphine and B-HT 920 produced large decreases in striatal DA overflow in IDM, SDZ 912, terguride, (-)-3-PPP and haloperidol caused decreased DA overflow. The close agreement with results from the cAMP assay suggest that the GBL and IDM tests may be useful for estimating the DA agonist intrinsic activity, while the IDM test does not discriminate between weak partial agonists.

726.16


S 21357-1 (Trihydrocholine of 3-(2,4,6-tetrafluorophenyl)-N-methylpropenamide) has been shown to be a potent and selective NMDA receptor antagonist in vitro with high selectivity for the NMDA (Kb = 2.3 nM) and non-NMDA (Kb = 25-46 nM) receptors and with low affinity (Kb = 1000 nM) to the NMDA, α and β-agonists. S 21357-1 was evaluated in the anxiodyne test battery which may be able to assess escape and defensive reactions of a mouse after exposure to a predator (i.e. a rat). The animals were tested in the oval runway in two situations: after exposure to the predator stress and injection of different doses of our putative anxiolytic treatments. Two doses of 0.125 and 0.5 mg/ml were tested (respectively 20% and 10% of the time spent in the open compartment with a possible range of 0-100%) and the presence of the predator reproducing a panic attack. In the first situation, S 21357-1 was able to prevent the increase of the jump escapes induced by the stress and the stress-induced decrease of wall rears at the doses of 0.125, 0.5 and 2 mg/ml, in addition, 32-50% reduced the increase of the wall climbs only at the lowest dose. In the second situation, S 21357-1 also reduced the escape behavior: the number of avoidance was reduced at the doses of 0.125 and 0.5 mg/ml (respectively 19% and 14%), as well as the avoidance distance (94% at the doses of 0.125, 0.5 and 2 mg/ml, respectively). The forced contact, S 21357-1 significantly decreased the frequency of walking towards the rat at 0.125, 0.5 and 2 mg/ml, respectively 13%, 21% and 10% (p<0.01). The time spent in the open compartment exploratory in mice in dose-dependently increased the number of transitions in the oral doses of 0.25, 1 and 4 mg/ml respectively (p<0.02, p<0.04, p<0.001) and the time spent in the open compartment with a possible range of 0-100% and the presence of the predator reproducing a panic attack. The effects of S 21357-1 showed anxiolytic-like activity in the compartment exploratory in mice: it dose-dependently increased the number of transitions in the oral doses of 0.25, 1 and 4 mg/ml respectively (p<0.02, p<0.04, p<0.001) and the time spent in the open compartment with a possible range of 0-100% and the presence of the predator reproducing a panic attack. These results suggest the interest of S 21357-1 as an antipsychotic-like and anxiolytic-like agent with minimal side effects.

727.1

THE DELAYED ANTICHALFECT EFFECT OF MK-801 IN RATS: SIGMA VERSUS NON-SIGMA (5-HT1A) RECEPTOR BLOCKADE. S.C. Lee and B.L. Commissaris, Dept. of Pharmaceutical Sciences, Wayne State Univ., Detroit, MI 48202 U.S.A.

The present studies examined the receptor mechanism for the delayed anticholinetic-like effect produced by diazoxide (MK-801) in Conditioned Suppression of Trialling (CST) conditioned conflict paradigms. Two questions were addressed: [1] Do other agents which reduce human MNS neurotransmission be antagonistic to MK-801 in the CST? [2] What treatment(s) can block the MK-801 effect? In Experiment 1, female rats were tested in the CST conflict task using a multiple within- day testing schedule (3 conflict sessions/day: 2 hrs/session). A thorough (0–60 hours in 6-hour interval) dose-effect of MK-801 and a variety of agents which reduce human MNS neurotransmission was determined. MK-801 (4 mg/kg, IP) exerted a delayed anticholinetic effect with a latency to onset of approximately 12 hours and a maximal effect at approximately 24 hours. In contrast, other MNS antagonist exerted a delayed anticholinetic effect, although CPP and AP-D did produce modest anticholinetic effects at a short interval (i.e., 40 minutes) pretreatment interval. In Experiment 2, the effects of 0.2 mg/kg MK-801 were selectively antagonized by the sigma agonist quinpirole (0.1 mg/kg, IP) in contrast to the anticholinetic antagonist mecamylamine (0.2 mg/kg, ICV), the opiate agonist naloxone (1.0 mg/kg, IP) and the benzodiazepine antagonist Ro-15-1788 (0.5 mg/kg, IP) did not antagonize MK-801. Together, the delayed anticholinetic effect of MK-801 may due to its action at MNS, sigma, and D2 receptors. (Supported in part by NIMH 41171).
277.3
EFFECT OF CARBAMAZEPINE ON PREGNENOLONE SYNTHESIS IN C57 GLIOMA CELLS AND RAT BRAIN
E.S. Park, K. Kozuma**, H.K. Mohri, and W.J. Potter. Section of Clinical Pharmacology, NHL, Bldg 10, 8th 2046, Bethesda, MD, 2002

Although carbamazepine (CBZ) has proven effective in the treatment of both neurological and psychiatric disorders, its mechanisms of action remain unclear. We have begun to explore the hypothesis that CBZ may affect the brain levels of pregnenolone, a neurosteroid known to modulate GABA receptor in CNS. In the present study, cultured C6 glial cells were incubated with CBZ at concentrations of 1 to 100 μM for 0 to 120 min. After the incubation period, the samples were extracted with ethyl acetate. Pregnenolone was separated by HPLC, and quantitated using a radioimmunoassay method. The results suggest that CBZ can increase pregnenolone content in rat brain tissue at concentrations used therapeutically in humans. This finding may provide novel insights into the mechanisms underlying one or more clinical effects of CBZ.

277.4
DESENSITIZATION AFTER A SINGLE DOSE OF THE 5-HT1A RECEPTOR AGONIST FLESNOXAN UNDER BASAL AND DEFENSIVE-BURYING CONDITIONS.
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A single injection of a 5-HT1A receptor agonist has been shown to attenuate 5-OH-DPAT-induced corticosterone secretion in the rat (Kelder and Ross, N.-S. Arch. Pharmacol. (1992) 546:121). In order to assess whether this also implies behavioral desensitization, we studied the effects of a single pretreatment with flesinoxan under basal conditions and in the situation of the defensive-burying paradigm. Male rats received an anxiolytic dose of flesinoxan (3 mg kg⁻¹ s.c.), which suppresses defensive burying, or vehicle on day 1. Plasma corticosterone and glucose concentrations were determined on day 2 or day 6, 60 min after a second flesinoxan or vehicle treatment. On day 2 only, drug-induced increases in corticosterone and glucose under basal conditions were found to be diminished after flesinoxan pretreatment. No effect of pretreatment on lower lip retraction was observed. In the defensive-burying condition, flesinoxan pretreatment on the previous day also attenuated plasma corticosterone and glucose elevations. It is suggested that desensitization related to hormonal effects of 5-HT1A receptor agonists involves a mechanism distinct from that underlying other symptoms of 5-HT1A receptor activation.

277.5
ANTAGONISM OF AMPHETAMINE-INDUCED BEHAVIORAL CHANGES IN SELECTED MEMBERS OF PRIMATE SOCIAL COLONIES IN LOW DOSE APOMORPHINE.

Low doses of the dopamine (DA) agonist apomorphine (APO) have been shown to lessen psychotic behavior in schizophrenic patients. This activity appears to be related to action on the pre-synaptic DA receptor resulting in decreased DA release. Recently, there has been interest in developing agents with this property as antipsychotic candidates. In this study, we tested the effect of low dose APO on amphetamine (AMPH)-induced behavioral changes in monkeys that appear to have face validity or correlated validity to human psychotic behavior. The effect of APO was compared to the antipsychotic disulfiram (DISU) and flesinoxan (FLX).

The subjects were 4 females of a stable social colony of 5 adult stump-tail macaques (Macaca fascicularis). The study followed a cross-over design with 2 monkeys receiving drug per treatment day. Following observation of normal behavior (baseline), d-AMPH, 1.0 mg/kg, was given i.m. 15 min before observation for 2 days. Then, either APO, 0.1 mg/kg, was administered i.m. 15 min or CLOZ, 5 mg/kg, a 2.5 hr before for observation for 2 consecutive mornings and in the afternoon of day 1. APO was given as above. On each day, a "blind" observer quantitated and recorded the behavior of each animal using a checklist of 40 social and solitary behaviors. Alone, AMPH significantly increased submissive gestures, checking (visual scanning), induced stereotypy, and eliminated initiated social grooming. APO antagonized AMPH-induced submissiveness, stereotypy, and restored social grooming, whereas CLOZ blocked AMPH-induced submissiveness and checking. Neither APO or CLOZ induced movement abnormalities, but CLOZ-treated monkeys had a significant increase in resting. The results of this study suggest that this paradigm may be valuable in screening DA partial agonist as potential antipsychotic agents.

277.6
In Vivo Electrophysiological Effects of ABT-418: A Novel Cholinergic Channel Activator (CSCA): E.J. Ruda*, C.A. Briggs, P.S. Aronica, Neuroscience, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL, 60064-3500

ABT-418((S)-3)-methyl-(S)-(−)-2-pyrrolidinylxoxazole) has in vitro nicotinic-cholinergic functional and receptor binding properties (Ameret, et al., J Pharmacol Exp Ther, in press), that, in conjunction with animal behavior studies (Decker, et al., J Pharmacol Exp Ther, in press), suggests a more selective and less toxic action compared to nicotine. Nicotine itself desynaptizes neocortical EEG while also suppressing paroxysmal spike wave discharges. This study evaluated the effects of ABT-418 on these neocortical EEG parameters that are affected by nicotinic acetylcholine (nAChR) receptor activation.

Six and 12 month Wistar rats were surgically implanted with recording electrodes over the frontal and parietal cortices. ABT-418 (0.62, 1.9, and 6.2 μmol kg⁻¹ i.p.) did not produce any significant change in FFT-analyzed EEG activity in unanesthetized 6 month rats. In contrast, at all tested doses of (-)-nicotine (0.19, 0.62, 1.9 μmol/kg, i.p.) significantly (p<0.05) lowered 1-15 Hz total power values. ABT-418 (1.9 and 6.2 μmol kg⁻¹ i.p.) significantly lowered the incidence of spontaneous 6-10 Hz neocortical spike wave discharges in awake 12 month rats. This effect was inhibited by the cholinergic channel blocker mecamylamine (5.0 μmol/kg, i.p.) indicating that ABT-418 attenuation of spike wave activity is mediated by nAChRs.

The relative lack of ABT-418-induced neocortical desynchronisation distinguishes this compound from nicotine. This study suggests that the attenuation of spike wave discharges and the reported behavioral effects of ABT-418 are not a simple consequence of the neocortical activation.

277.7
SEROTONERGIC OVERACTIVATION, LIKE DOPAMINERGIC OVERACTIVATION, DISRUPTS CROSS-MODAL SENSORY GATING AS MEASURED BY AUDITORY AND VISUAL PREPULSE INHIBITION IN RATS. L.L. KERIN**, T.C. McCloskey, R.A. Padich, V.L. Taylor, & C.J. SCHMIDT. Marion Merchel Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, Ohio 45215.

Dopamine (DA) and/or glutamate (GLU) imbalances are generally thought to contribute to schizophrenia's symptomatology. Increasing evidence suggests a possible role for serotonin (5-HT) as well. Prepulse inhibition (PPI), a measure of sensory gating, is disrupted in schizophrenics, and by psychotomimetics in rats. In the present study, cross-modal PPI of acoustic startle in Wistar rats was produced by using relative auditory stimulus (sound) or a visual stimulus (light) as the prepulse. DA overactivity induced by d-amphetamine or apomorphine, or GLU overactivity produced by the cation channel blocker D-600 (PCP) or MK-901, disrupted auditory and visual PPI. MDL 100,455, a competitive NMDA antagonist, decreased baseline without markedly disrupting PPI. The 5-HT releaser fenfluramine (5.0 mg/kg) disrupted PPI, and these effects were attenuated by the 5-HT uptake blocker MDL 28,618A, or by central 5-HT depletion produced by the 5-HT reuptake blocker fluoxetine (30 mg/kg) i.g. injected two hours prior to testing. These findings indicate that excessive 5-HT overactivation, like DA overactivation or GLU overactivity, can disrupt auditory or visual sensory gating processes. The findings with PCA also suggest the utility of PPI as a tool to evaluate functional deficits accompanying CNS 5-HT neurotoxicity.

277.8
USE OF NON-PERMEABLE MICROCARBON BEARERS IN ALKALI METAL ION TRANSPORT NMR STUDIES OF PERFUSED NEUROBLASTOMA CELLS: C.Zachariah*, and D.Freitas. Dept. of Chemistry, Loyola University of Chicago, Chicago, IL 60626.

In Nuclear Magnetic Resonance (NMR) studies of cells in suspension, it is possible to distinguish between the chemical shifts of the intracellular and extracellular Li⁺, Na⁺, K⁺ and Rb NMR resonances by the use of anisotropic shift reagents (SR) in the extracellular medium. In NMR studies of cells anchored on microcarrier beads, which are permeable to alkali metal ions and/or SR, the intracellular resonance may overlap that of the internal bead volume. Cell-free suspensions of four types of beads which differ in size, ion permeability, matrix composition, magnetic susceptibility and charge distribution were used in this study. Cytolex I and CultiSpher-G beads in buffered solutions gave rise to two separate Li⁺ and Na⁺ resonances, while only one Li⁺ and Na⁺ resonance was observed in suspensions containing Biosil or glass beads. Hence the physical basis for the discrimination between the alkali metal NMR resonances in cell-free suspensions of Cytolex I and CultiSpher-G is the cation and anion permeability differences for NR transport experiments of human neuroblastoma cells anchored on impermeable Biosil beads will be presented. The relevance of these studies to understanding the mechanisms of lithium action in the treatment of manic depression will be discussed.
HYDROFLUORIC ACID ENHANCES RECOVERY FROM ISOFLURANE ANESTHESIA IN RATS: BEHAVIORAL AND PHYSICOCHEMICAL MEASURES. R. Del Vecchio, A. Bandel, C. C. Huang, and R. N. Renshaw, Dept. of Biological Psychology, School of Medicine, Brown University, RI 02912.

Preliminary results indicate that when isoflurane (1) with trace amounts of hydrofluoric acid (HF) (50% recovery of plasma) was administered to rats, the recovery appeared to be faster than that of control animals. Therefore, we investigated the effect of adding HF to isoflurane. 50% of animals survived to receive HF. The results showed that the recovery of animals treated with HF was faster than that of control animals. The results also indicate that the addition of HF to isoflurane can enhance the recovery of animals from isoflurane anesthesia.

DEGENERATIVE DISEASE: ALZHEIMER’S—MODELS, ASSESSMENT, AND TREATMENT


To study the sequence of neurodegenerative events which could be associated with cholinergic cell death in Alzheimer’s disease, rat septal cholinergic neuronal cultures were exposed to chronic excitotoxic stress by glutamate. Counts of ChAT immunopositive cells and measurement of CA1 activity revealed that concentrations of glutamate on the order of 700 μM killed 50% of cholinergic neurons after 24 h of treatment. This toxic effect was not aimed at cholinergic neurons specifically, since other populations of cells present in these cultures were also affected. The non competitive NMDA receptor/chemoattractant antagonist, MK-801 (10 μM), abolished the neurotoxic effect of glutamate by preventing and rescuing from late degeneration both cholinergic and non cholinergic cells when concentrations of glutamate did not exceed 500 μM. The same degree of neuroprotection was afforded by the pyrimidyl antagonist, ficyclidine (2 μM). On the other hand, the kainate/nicatate receptor antagonist, CNOX, lacked protective effects. NGF used in standard culture conditions to enhance expression of the cholinergic phenotype was shown to inhibit glutamate toxicity. It should be noted that the presence of the NO synthase enzyme in many of the cholinergic neurons did not confer to these cells a particular resistance to glutamate toxicity. Furthermore, the fact that the NO generating agent, sodium nitroprusside, was capable of mimicking some of the effects of glutamate, the lack of protection of various NO synthase inhibitors (L-NAME, L-NG, 30 μM) to glutamate toxicity suggests that NO does not participate to the toxic process as an intercellular messenger.

T27.10


YM-35992 ((-)–(+)–2-[[[(7-fluoro-4-indanyl)oxy]methyl]morpholine] mono-hydrochloride) possesses affinities for not only serotonin (5-HT) uptake site (Ki=21 μM) but also 5-HT, receptors (Ki=104 nM), and has a relatively weak affinity for tricyclic antidepressant (Ki=247 nM), (Ki=104 nM), respectively. YM-35992 inhibited on a unit dose of 5-HT uptake sites (Ki=142 μM) and 5-HT, receptors (Ki=38 μM). Does not show any interactions with 5-HT uptake sites at a dose of 60 μg/kg, i.p., respectively. On the other hand, YM-35992 has an affinity for 5-HT uptake sites (Ki=142 μM) and 5-HT, receptors (Ki=38 μM). YM-35992 (10 mg/kg, p.o.) significantly reduced the immobility time in the mouse tail suspension test, but trazodone conversely prolonged the immobility time in a dose-dependent manner. Interestingly, both YM-35992 and trazodone ameliorated the learning impairment in olfactory bulbectomized rats after an acute administration, but chlorpromazine did not. In this model, both antidepressants are known to ameliorate the learning impairment only after chronic but not acute administration. These results suggest that YM-35992 would be a novel type antidepressant with more potent effects than trazodone and earlier onset of action than conventional selective serotonin re-uptake inhibitors.
Animal model of wandering in Alzheimer’s disease: Neural changes in proximal and distal sites following colchicine lesions of the dorsal hippocampal formation. J.R. Ryan and I.P. Ryan
Neurobehavioral Research Lab, Psychology Department, SUNY Plattsburgh, Plattsburgh, New York 12901.

The neuroanatomical and neurobehavioral effects of wandering in Alzheimer’s disease (AD) have been virtually unexplored despite the problematic nature of the behavior. Ryan and colleagues (1990, 1993) have investigated the behavioral profile of the hippocampal system. The present study investigated changes in the hippocampus and related structures following dorsal hippocampal formation lesions. Twenty-four Long-Evans hooded rats received either bilateral injections of one of three colchicine doses (10 μg, 15 μg, 25 μg) or artificial CSF into the dentate gyrus and/or CA1 regions. Ten days following surgery, the animals were behaviorally tested in the activity chamber for attention and exploration followed by four consecutive days of T-maze testing for spontaneous alternating behavior. Increased activity, decreased attention and perseverative behavior were indicated in rats with marked cell loss in the dorsal dentate gyrus and CA1. The study supports the hypothesis that deficits in spatial memory characterized the animal model of wandering in AD are exacerbated by perseverative behavior and hyperactivity.

728.6 CORPUS CALLOSUM PATHOLOGY IN ALZHEIMER SUBJECTS: DISRUPTION IN INTERHEMISPHERIC TRANSFER. Y. Latkoczy1, F. Lepori2*, S. Gauthier1 and M. Lepage1 1 Groupe de Recherche en Neuropsychologie Experiente, Universite de Montreal et 2 Centre McGill pour Etudes sur le Vieillissement, Montreal, QC, Canada.

The main feature of Alzheimer’s disease (AD) concerns the neuropathological and physiopathological changes in the associative areas of the cortex. Recent studies have demonstrated that besides the grey matter, the white matter of the cerebral cortex, which includes the minor and major forceps of the corpus callosum (CC), is also severely affected in AD. This might be in part a result of the degeneration of the pyramidal cells within these areas which furnish most of the commissural fibers. Fibers of the CC link the two hemispheres following a rostro-caudal topographical distribution. Thus, the motor fibers of the CC are mainly found in the genu, the somesthesis fibers occupy the trunk, the auditory fibers cross in the isthmus and the visual fibers project through the splenium. The aim of this study was to evaluate interhemispheric transfer in AD subjects and hence to assess the integrity of the CC. Several tasks, including tactile discrimination, tactile and visual discrimination, simple reaction time to lateralized flashes of light or to puff of air, bimanual coordination, etc., which attempted to test interhemispheric transfer within these systems were administered to 10 AD and 10 matched controls. Results showed that inter- but not intrahemispheric performance was significantly impaired in AD relative to controls. This finding is interpreted as showing that AD affects not only the well established memory and cognitive functions but also interhemispheric integration.


The practice of diagnosing Alzheimer’s disease (AD) without classification as to age of onset is under increasing challenge. Recent reports of genetic disimilarity between early and late-onset AD patients provide qualitative support for previously noted differences. These observations span clinical, neurobiological, and neuropsychological parameters. As the classic pathology of AD has yielded to observations that the substantial histological similarities, once used to unify early- and late-onset patients, segregate the groups in terms of quantity and distribution. To assess the validity of an age-based dichotomy, published data supporting and refuting the argument were reviewed. Conclusive claims cannot be made from a review of small patient cohorts; yet, a substantial amount of data differentiate early- and late-onset AD. Among them, evidence for differences in disease dynamics, language, cholinergic deficits, and metabolic function are strongly supportive of heterogeneity in AD identified by age of disease onset. These observations are reviewed and their implications for continuing AD research are explored.

728.8 ASSOCIATION BETWEEN MOTION SENSITIVITY DEFICITS AND DIFFERENTIAL LUMINANCE SENSITIVITY IN SENILE DEMENTIA OF THE ALZHEIMER’S TYPE. A. Belsieguil1, G. L. Test2*, P. Morin2, M. Wolff and M. Pellegrin. 1University of Montreal, Montreal, H3C 3J7, Canada, 2Washington University, St Louis, MO 63110 and 3Henry Ford Hospital, Detroit, MI 48202.

The visual abnormalities associated with senile dementia of the Alzheimer’s type (SDAT) include both motion sensitivity deficits and reductions in differential luminance sensitivity (DLS). The principal objective of this study was to quantitatively determine the relationship between these deficits in patients with early to mid SDAT. Random dot kinematograms (RDKs) were used to assess global motion sensitivity in 16 patients with diagnosed SDAT and 19 visually and cognitively normal control subjects of similar age. Motion sensitivity testing was conducted twice within a month on each participant and the results of the two tests were averaged. DLS was evaluated with automated static perimetry (Humphrey, 30-2). Only participants with reliable visual fields (by manufacturer’s criteria) were included. The global sensitivity, indices of visual field sensitivity (i.e., mean sensitivity, 90% probability (PSD)), and corrected PSD (CPSD) were used to quantify differential luminance sensitivity. Motion sensitivity was reduced significantly in SDAT patients compared to the controls (p<0.001). DLS was reduced significantly (i.e., significant visual field defects were detected) in 11/16 SDAT patients, MD, an index of generalized reduction in DLS, was significantly correlated with motion thresholds (p<0.01). Motion sensitivity was significantly correlated with global and differential luminance sensitivity deficits in patients with SDAT. In addition, the correlation between motion and luminance sensitivity suggests a link between these two deficits. (Supported in part by NIH grant A050681)

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728.11

Alzheimer's disease (AD) is divided according to DSM-IV into mild (score between 6) and severe groups. There are many genetic, neurobiological, and clinical differences among AD patients. Early-onset AD patients may differ from their peers in their symptomatology. Thus, different therapeutic approaches may be considered for early- and late-onset AD patients. Several clinical trials with acetyl-l-carnitine (ALCAR) have been conducted in AD patients in the past years. Ten studies were reviewed to look retrospectively at the effect of ALCAR in the AD patient. Treatment was placebo-controlled Double-blind, clinical studies with ALCAR in patients diagnosed with AD were pooled in a comprehensive meta-analysis. For each study, cognitive and functional outcomes measures for efficacy were evaluated. By using standardized ranges for treatment comparison on cognitive tests, ALCAR-treated early-onset patients tended toward slower progressive loss (p < 0.01) than placebo-treated subjects, whereas using logrank scores the effect of ALCAR achieved significance (p < 0.05). Functional tests showed a treatment effect favoring ALCAR by using standardized ranges (p < 0.05). These data suggest that ALCAR may affect the clinical progression of AD.

728.12

Vasoactive intestinal peptide (VIP) has been shown to be involved in the promotion of neuronal survival as well as in acquired memory and learning. We now demonstrate that a superactive lipovapic VIP agonist inhibited neuronal cell death in an Alzheimer's model in vitro and accelerated the formation of cholinergic synapses in an in vitro Alzheimer's paradigm. Thus, addition of 250 nM of a fragment of the beta-amylloid peptide to rat cerebral cortical cells in vitro resulted in a significant 70% cell death which was attenuated by co-treatment with stearyl-Nle-VIP. Furthermore, in an in vivo Alzheimer's model of rats treated with the cholinergic block of AF64A, which induced an impairment in spatial learning, stearyl-Nle-VIP injected i.o. or i.v. alleviated the impairment characteristic of Alzheimer's disease. These data support the neuroprotective intervention as a therapeutic strategy for neurodegenerative diseases.

We thank Dr. R. L. Berman for the initial sample of AF64A. Supported by Fujimoto Corp. Japan.
DEGENERATIVE DISEASE: PARKINSON'S—HUMAN STUDIES

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DEGENERATIVE DISEASE: PARKINSON'S—HUMAN STUDIES


Patients with Parkinson's disease, age-matched controls, and young control subjects performed fast, discrete elbow and wrist flexion or extension movements in a sagittal plane using the index finger to move one of the joints "as fast as possible." Relative stability in the other, postural, movements is more variable in the patients and in both control groups. Typically, EMG patterns in muscle pairs acting at both joints displayed a commonly observed tri-phasic pattern. During both elbow and wrist movements, in both flexion and extension, the elbow flexor and the wrist flexor demonstrated similar, synchronized EMG patterns, while the elbow extensor and the wrist extensor also showed similar patterns of activation. A cross-correlation analysis of the EMG confirmed virtually simultaneous bursts in the wrist and elbow flexors and in the wrist and elbow extensors. In all three groups, there were no signs of anticipatory activation in any movement in about 90% of movements. The analysis of a simple biomechanical model has suggested that simultaneous EMG bursts may be used to minimize the displacements in the postural joint. We assume the anticipatory activation is not a separate process, but a separate peripheral pattern of a single control process that may involve a number of joints and muscles. The observed relation between the EMG patterns is apparently a universal synergic system which is preserved in elderly and in Parkinson's disease. We conclude that the postural deficits in Parkinson's disease are not related to a basic deficit in feedforward postural control but to other factors that may include the specificity of maintaining the vertical posture.

729.7 ALLEVIATION OF MOTOR SIGN OF PARKINSON DISEASE AFTER STIMULATION OF THE INTERNAL SEGMENT OF THE GLOBUS PALLIDUS: EXPERIMENTAL EVIDENCE IN HUMAN

Methods: a bilateral sub-threshold stereotactic needle implantation of the globus pallidus substantia nigrae has been previously shown in monkeys and human. In the present work, we studied the effect of HFS of GPi in five Parkinsonian patients with advanced stage disease and with associated rigidity. Patients between 40 and 60 years old, were at Hoehn and Yahr stage 3 to 5 and suffering from Pharmacological resistant form of the disease. Patients were treated with dopamine agonists, "tapping" test and before the electrode implantation in 6 different situation: one week before the operation 3 weeks and 3 weeks after the implantation with Dopatherapy, and 3 weeks and 4 weeks without stimulation; and without Dopatherapy, and 5 weeks or 6 weeks without. Monopolar electrode was stereotactically implanted using the stereotactic Leksell atlas and MRI. Results showed that for stimulation sites situated at the base of the GPi, the stimulation with 3 Hz, toward the head, reduced rigidity and turning movements (AD) and increased motor activity in the patients. The effect was more prominent in the arm. The effect was maintained for 3-6 months post surgery. We conclude that the stimulation of the globus pallidus substantia nigrae is effective for the motor signs of advanced Parkinson's disease without overt surgical complications.

729.8 SPATIAL LEARNING DEFICITS IN PARKINSONISM

R. J. Arcenta*, D. J. Ingle, and L. Cote, Neurological Institute, Columbia-Presbyterian Medical Center, West 168th St., New York, N.Y. 10032.

Ingle and Thompson (1993) showed that Parkinson Disease (PD) patients of moderate severity performed poorly on a spatial memory task after waking 4 to 8 meters forwards. Recently Arcenta, et al. replicated the post waking deficit in a new group of PD patients. In the present study we measured localization memory linked to arm movement, rather than to locomotion. Here the blindfolded subjects (S) point to a remembered target (T) on the table before them using the tip of a bent index finger held by one hand at the other end. All Ss had the opportunity to learn the location of the bent wire's tip in relation to the hand during a two minute period of touching the tip to various table locations. Testing was done with both the bent wire and the index finger. When 14 PD and 13 controls were tested, the PD Ss were not significantly worse than controls in localization (T by memory) using their index finger, but were significantly worse in learning to point with an artificial extension of the hand. The control errors averaged 73 mm each while the PD patient errors averaged 96 mm each. We speculate that spatial memory linked to locomotion and to arm movement are mediated by the caudate nucleus and the putamen respectively.
729.9
REVERSAL OF CATECHOL BY STRESS: INVOLVEMENT OF THE MESOCORTICAL BUT NOT THE MESOLIMBIC SYSTEM. L. Hernandez*, T. Tunc*, E. Murai, and T. Takahashi. Laboratory of Neurochemistry, Medical School, Los Andes University, Mérida 5101, Venezuela.

Stress reverses catechol in Parkinsonian patients presumably by releasing dopamine. In an experimental model, i.e. haloperidol induced catechol was re-versed by forced running (F). DA activity was assessed by microdialysis of the prefrontal cortex (PFC) or the nucleus accumbens (NAC). In rats, each region (PFC or NAC) one group (HAL+SWM) received an ip haloperidol injection (5 mg/kg) and was forced to swim, another group (HAL) had not forced to swim and a third group (VEH+SWM) received vehicle and was forced total (F). After haloperidol the rats exhibited catechol and dopamine and its metabolites increased. FS increased DA activity in the PFC too. However, the increase was significantly greater in the PFC of the HAL+SWM than in the HAL or the VEH+SWM groups (F2/196=22.78, p<0.01). No difference of DA activity in the NAC was observed when the HAL+SWM vs the HAL group were compared (F1/112=3.01, NS). Another three groups of rats received microinjec-tions of DA in the PFC during haloperidol induced catechol. Intracortical SA injections abolished catechol (F1/11=34.8, p<0.001). These experiments suggest that DA release in the PFC contributes to the reversal of catechol by stress.

729.11

[1-123I]-CIT (25-carboxymethoxy-3-hydroxy-iopropyl)tracers monoamine transporters were located on the terminals of dopaminergic projections from the substantia nigra to the striatum providing a marker for neurons which degenerate in Parkinson’s disease (PD). [1-123I]-CIT SPECT imaging performed in 8 patients with hemi-Parkinsonian symptoms and in 8 age and sex-matched healthy subjects. All patients had very mild symptoms in the affected side (Hoehn and Yahr stage 1/11) and had not received treatment with L-Dopa.

Data was analyzed relative to two outcome measures: ratio of specific non-specific striatal activity and striatal uptake expressed as percent injected dose. Disease stage and severity was assessed by Hoehn and Yahr Staging and UPDRS scales. Specific non-specific striatal binding ratios in PD patients contralateral (2.7±9) to the symptomatic side were reduced to 47±% (p<1.1) to the symptomatic side were reduced to 68% of HS ratios (5.7±1). Striatal activity in PD patients expressed as percent injected dose was reduced to (55%) of HS (with no difference in occipital uptake). The reduction in striatal activity in the PD patients both in the symptomatic and the effectively pre-symptomatic striatum suggests imaging of dopamine transporters with [1-123I]-CIT may be a useful tool for early diagnosis of PD and possibly for ‘pre-symptomatic’ mesencephalic dopaminergic degeneration.

729.13

We have previously shown that Alzheimer’s disease (AD) cases with a Parkinson-like syndrome (AD/Park) show significant (50-80%) losses of TH immunoreactivity (IR) in the substantia nigra (SN) and pars reticulata of the substantia nigra pars compacta (SNpc). We have now shown that levels of TH mRNA in this region are also reduced in AD/Park cases compared to normal controls (NC). The SNpc is an area of the SN most affected in PD so reduced levels of TH mRNA in AD/Park cases suggest that there are also changes in the expression of TH at the mRNA level. In the SNpc we have also seen decreased levels of DAT mRNA and increased levels of DAT protein expression. The SNpc is the important site for transporter studies and the present findings suggest that there are changes in the expression of DAT at the mRNA level.

729.12

We measured regional cerebral blood flow (rCBF) with positron emission tomography (PET) and [15O]water before and after acute administration of levodopa (in combination with carbidopa) in 12 patients (5 females) with Parkinson’s disease (PD), Hoehn and Yahr stages 1 and 2, and 5 age-matched normal women (NL). All subjects were pretreated with 200 mg of carbidopa about 2 hrs prior to PET. Regional CBF was measured prior to levodopa and 75 minutes after taking p.o. levodopa 150 mg with carbidopa 37.5 mg. Images were transformed into standard stereotactic space. We split both the PD and NL groups into 2 subgroups to permit response identification and subsequent confirmation in each group. Levodopa produced discrete foci of activation in the range of 5 to 15 s above normalised mean global flow. In the PD group, there were confirmed responses in bilateral midbrain, right frontal cortex and left putamen. Right putamalar response only reached p<0.1. In the NLs, there were confirmed responses only in right midbrain and right frontal cortex. The relatively low f for NLs limited the power of response confirmation. The bilateral midbrain responses in the NLs were significantly greater than in the PD group (p<0.04 for MANOVA of bilateral midbrain, bilateral putamen and right frontal cortex responses for NL and PD; F: pos hoc t-tests p<0.05 for right and left midbrain). There were no other statistically significant differences between NL and PD. We believe that levodopa-induced blood flow responses provide a powerful tool for investigation of functional changes in dopaminergic neurons and their connections.

729.10

Parkinson’s disease (PD) is characterized by a massive degeneration of the dopaminergic neurons containing in the midbrain. However, the vulnerability of these neurons is heterogeneous both across different subregions of the substantia nigra pars compacta (SNpc), the most affected structure in this disease. To determine the exact pattern of cell loss, it is necessary to have landmarks independent of the degenerative process to subdivide the SNpc. We have defined a dopamine receptor (D2) imaging protocol (DBP) in the midbrain. The DBP was validated in the monkey and in the human post-mortem samples. The DBP was performed in 3 groups of patients with PD: non-motor (+M3), motor (+M4) and severe motor (M5) PD.

The DBP-positive neostriatal tracer in the ventral midbrain survived in the PD brains. Moreover, although the DBP-negative zones were spared, their two-dimensional organization was similar to that seen in the control midbrain. We conclude that DBP imaging patterns could be used to calculate very precisely the relative loss of dopamine-containing neurons in the different parts of the SNpc. Supported by NIH Awards RO1NS02529 and Lavoisier Grant (French Foreign Office).

729.8

There exists neuropathological and biochemical evidence indicating that the serotonergic system is affected in Parkinson’s disease. The alterations observed include the presence of Lewy bodies in 5-HT cells in the dorsal raphe, a loss of more than 50% of large neurons in the nucleus, and a decrease in the levels of 5-HT and its metabolites 5-HIAA in the cortex and basal ganglia. In addition, serotonin transporter (5-HT1) binding in the 5-HT transporter (5-HT1) and the 5-HT3 receptor in the dorsal raphe of control and Parkinsonian patients using 5-HT1-citrapram and 5-CT-DPAT, respectively.

The binding of both ligand’s serotonin-receptor system was not significantly different from control levels. This contrasts with the reported decreases in 5-HT transporter binding in forebrain regions and loss of 5-HT cells in the dorsal raphe of Parkinsonian patients. Our results suggest that the remaining neurons overexpress the 5-HT transporter in order to compensate for cell loss. We thus examined the mRNA encoding the 5-HT transporter in situ hybridization using a 5-HT-labeled oligonucleotide probe. A strong hybridization signal was observed overlying cell bodies in the dorsal raphe. In the case of 5-HT1 receptors, the mRNA content per cell was higher in Parkinsonian patients compared to normal tissues. These results might reflect an enhanced expression of 5-HT transporter mRNA. The types of cells expressing this mRNA remains to be determined.
Mental Illness: Depression, Anxiety and Schizophrenia

**370.1** 

OBX rats simulate aspects of chronic Major Depression in humans, e.g., appetite, learning deficits, sleep and sexual behaviors, decreased corticosterone plasma. These are not caused by anoxia and are usually reversed by antidepressant treatments, including ECT. ECT inverts the pattern of changes. The animals with OBX show behavioral effects that mimic the effects seen in OBX rats. OBX rats were treated with vanadate, a known antidepressant, and showed significant improvement in behavioral measures. The results suggest that OBX rats may be a useful model for studying the effects of antidepressants on the behavior of depressed individuals.

**370.2** 
**A Rat Model for Mania: Relation to Brain Amines.** R.S. El-Mallakh, P.L. L-T. Harrison, D.G. Changard, and B.S. Levy*. Dept of Psychiatry, Biochemistry, Neurology, and Laboratory of Biological Psychiatry, University of Louisville School of Medicine, Louisville, KY 40292.

We previously have described a rat model of mania that has been used in a number of studies and has been characterized through preclinical and clinical investigations. The model is based on the administration of a combination of drugs, including reserpine, amphetamine, and desipramine, which produce a range of behavioral and biochemical changes in the rat. In this study, we examined the effects of these drugs on the levels of brain amines and their metabolites in this model. Our results indicate that the model is a useful tool for studying the mechanisms of mania and for developing new treatments.

**370.3** 
**Magnetic Resonance Imaging and Brainstem Evoked Response in Geriatric Depression.** B. Kalpers, S. Budzilin, R.C. Yung* and O. Akefopoulos. The New York Hospital - Cornell Medical Center, 21 East 64th Street, New York, NY 10065.

Introduction Rate-dependent latency shift in brainstem auditory evoked response (BAER) is reportedly associated with occult brain pathology. Latency increases in BAER is elicited in elderly patients with a history of major depression was previously reported by our group. Preliminary findings are now reported that corroborates the latency change with findings on brainstem magnetic resonance imaging (MRI) in mild elderly depressed subjects. (t=7.1, p<0.01) reasons for failure of MRI and BAER. Psychiatric diagnosis was established using a structured clinical interview. Proton density axial images of the brain were classified using criteria proposed by the DSM-IV of Alzheimer's Disease. (CERAD) as normal, or with probable putative, or definite or white matter changes. BAER was performed using metallic stimuli of 1 to 7.1 μV and 80 μV clicks and seconds and the latency shift was not significant. Reduced latency shift in older patients with concomitant lesions and one patient with putative lesions of the lower brainstem had latency shift > 50 milliseconds. In this case depression was of the late-onset type. Among the remaining 11 patients, latency shift was < 50% and 8 of 11 had definite or white matter changes. BAER was performed using stimuli of 1 to 7.1 μV and 80 μV clicks and seconds and the latency shift in older patients with concomitant lesions was normal in two and brainstem imaging was normal in four. Depression was of the early-onset type in five of these six cases. Conclusion The above findings need further investigations and further sample size. The findings suggest that BAER are responsive to antidepressant drug treatment and relapse in the course of illness may be associated with brain morphologic changes. Brainstem imaging and BAER may prove useful prognostic tests in these patients.

**370.4** 

We previously reported higher postmortem cerebral cortical levels of the stimulatory G protein α-subunit, αs, in BD compared with control subjects (Young et al., J. Neuropsychiatry 6:135, 1994). Levels of αs and the inhibitory G protein α-subunit were also increased in MNL from BD but not major depressive disorder (MDD) subjects (Young et al., J. Neuropsychiatry 18:51, 1998). In the present study, the functional consequences of these protein changes were examined. MNL from BD and MDD subjects were examined for basal, GTPγS and forskolin-stimulated (100μM) AC activity. Subjects included depressed BD (N=9, age 31.4±4.8 (mean±SD); 40±5 years), age 65.7±22 and MDD (N=30, age 66.7±4.9 years) and age 72.7±2; MAM-D=1.±4.07) patients compared with age (35.2±25.9 and 35.5±4.6, respectively) and sex-matched healthy controls. In BD subjects, MNL membrane GTPγS- and forskolin-stimulated AC activity, expressed as specific activity, were significantly reduced (GTPγS=2.33, p<0.05; forskolin=2.3, p<0.001) as compared with controls. These findings suggest that GTPγS-stimulated AC activity was not significantly reduced (2.4%, 1.7, 0.3-0.05) and basal AC activity was not significantly reduced (0.8, 0.4) from that in healthy subjects. In comparison, basal membrane AC activity was significantly reduced as MDD subjects (39%, +3.75, p=0.027) compared with matched controls but GTPγS- and forskolin-stimulated AC activity did not differ. Thus, despite higher MNL αs levels in BD compared with healthy subjects, G protein-coupled AC activation is reduced in these cells contrasting with the possible enhanced functional status of this signal transduction process found in postmortem BD cerebral cortex (Young et al., J. Neuropsychiatry, 18:51). This difference, however, may be attributed to more effective compensatory adaptations in inhibitory control of AC activity and/or AC levels in MNLs than in cortex of BD subjects. (Supported by grants from the NAMI Stanley Foundation and MRC Canada.)
VOLUMETRIC STUDIES IN LATE LIFE DEPRESSION USING MRI. A.Kumar, D.Miller, L.Burke, W.Bell*, D.Evanko, S.Samuels, S.Gottlieb. Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104. The purpose of the study was to examine the volumes of ventricular, subcortical and whole brain cerebrospinal fluid (CSF) spaces in subjects with late life depression and to compare these indices to healthy age-matched controls and subjects with probable Alzheimer’s disease (DAT). Using a 1.5 tesla GE Signa scanner, we studied 24 subjects with late life depression (16 W, 9 M, Mean age 73.7 SD 7.46), 29 healthy controls (17 W, 12 M, Mean age 67.2 SD 8.45) and 34 subjects with DAT (17 W, 17 M, Mean age 66.4 SD 8.50). All depressed subjects met DSM-III-R criteria for major depressive disorder and had Hamilton depression scores of greater than 15 (Mean 19.7 SD 3.84). All subjects were medically stable and free of significant other neuropsychiatric disease. Volumetric density and T2 weighted images were used to maximize the contrast between brain and CSF. A semiautomated boundary program and segmentation algorithm were used to differentiate brain tissue from CSF. On normalized measures of ventricular and whole brain CSF, depressed subjects had larger volumes in both hemispheres when compared to the control subjects (p<0.01), as did the DAT subjects. When depressed and DAT subjects were compared, subjects with DAT had larger whole brain and right hemisphere CSF volumes and total and right hemisphere subcortical CSF volumes (p<0.05). These data suggest that widespread structural brain changes underlie late life depression and may be significant in the pathogenesis of mood disorders in late life.

EVIDENCE FOR INCREASED CONCENTRATIONS OF NEUROPEPTIDE Y IN PLASMA OF PATIENTS WITH PANIC DISORDER. S.Bougie*, J.P.Bouliener, J.Jerabeck, R.Leduque, F.Jolicoeur and A.Cadieux Departments of Psychiatry and Pharmacology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Quebec. C.Iida JH14 5N4. Recent experimental evidence suggests that neuropeptide Y (NPY) is involved in the modulation of anxiety-related behavior in rodents. Furthermore, NPY’s physiological effects are strongly associated with those of noradrenaline, a neurotransmitter involved in the expression of anxiety symptoms in humans both at the central and at the peripheral levels. The aim of the present study was to assess the relationship between NPY plasma concentration and anxiety levels in both normal volunteers (n=13) and patients (n=13) fulfilling the DSM-III criteria for panic-type disorders with or without agoraphobia. Plasma levels of NPY-Like immunoreactivity (NPY-LI) were measured in duplicate using a radioimmunoassay developed in our laboratory with detection limits being 7 pg/100 μl. The first results of this study suggest that, compared to normal controls, the baseline NPY-LI levels of panic disorder patients is increased (75 ± 10 pg/ml vs. 130 ± 8 pg/ml respectively). This difference was statistically significant, p<0.05. Supported by Fonds de la Recherche en Santé du Québec.

CARDIOVASCULAR, RESPIRATORY AND PARASYMPATHETIC ACTIVITY IN PANIC DISORDER. POST-TRAUMATIC STRESS DISORDER AND NORMAL CONTROLS. G.S. Gargani*, J.C. Sheem, D. Anti-Obeve, P. Andrew, B. Petti and A.J. Rais Dallas VA Medical Center and Department of Psychiatry, UT Southwestern Medical School, Dallas, TX 75216.

Although increased autonomic activity is observed in panic disorder (PD) and post-traumatic stress disorder (PTSD), it is unclear if autonomic pathophysiology is similar or different in these disorders. Heart rate (HR), systolic (SBP), diastolic (DBP), mean arterial pressure (MAP), respiratory rate (RR), indices of respiratory sinus arrhythmia (heart rate variability, HPV, heart rate, HR, and vagal tone, VT) were monitored while supine and for 10 minutes after standing in 19 normal controls (NC), 18 PD and 16 PTSD patients. The study included several trends for higher resting cardiovascular measures in PD and PTSD. PD patients had higher RR (NC: 15.3± 2.5 vs PD: 18.5± 2.5 , p<0.05) and a trend for a higher anoic gap and lower potassium than NC or PTSD patients. In response to standing, V wave and P wave were increased in NPY-LI patients with PD and PTSD. PD patients had higher HR responses than NC or PTSD patients. There was no significant group x time interaction in SBP only while other cardiovascular measures fell on a continuum between NC and PD. (2) PD patients, but not PTSD, had statistically significant group x time interactions in HPV and HR which showed a rebound increase 8 minutes into standing. These observations suggest that abnormal respiratory function may be specific to PD and that the type of abnormal cardiovascular and parasympathetic responses may be different between PD and PTSD.

Calcium dependency of the release of DA and 5HT from CNS slices from animals subjected to models of depression. E.H. Jaffe*, V. DeFries, C. Barra. Lab. Neurochemistry, IIVC, Apdo. 21827, Caracas 1020-A.

5HT and DA systems have been implicated in the physiopathology of depression. Previously (Neurosci. Lett. 162:157,1993) we showed an increased K stimulated release of 5HT from hippocampus (HP) and inhibition of the basal release from HP, n.accumbens (n.ac) and prefrontal cortex (PFC) of animals subjected to forst swim test and chronic isolation. Here we study the Ca dependency of the release of DA and 5HT from these 3 CNS structures. Slices were incubated in a static chamber system. DA and 5HT release measured by HPLC with electrochemical detection. K (30 mM) stimulated DA release was only significantly higher in n.ac of isolated animals and inhibited with Cd (150μM) a Ca channel blocker. 0.5-10mM Mg inhibited at the same degree release of control and isolated animals. A similar pattern was shown with the K stimulated release of 5HT from n.ac and Hip which were significantly increased in isolated animals, showing a greater inhibition with Cd than controls. Basal DA release was similar in control or isolated animals from the 3 CNS structures with a strong and significant inhibition of the release in 0Ca/Mg Krebs from isolated animals but not from control. A greater sensitivity to Ca blockade of the Ca dependent DA and 5HT release from isolated animals is suggested.

SODIUM LACTATE INFUSIONS IN AN ANIMAL MODEL OF PANIC DISORDER: FINDINGS OF ADOLESCENT P. Sheil*, S.R. Keim, J.R. Simom and W.J. McDermite, Dept. of Psychiatry, Indiana University Medical Center, Indianapolis, Indiana 46202. GABA receptor blockade in the dorso medial hypothalamus (DMH) of rats elicits a panic-like response. Our preliminary studies have shown that rats that have inhibition of GABA synthesis in the DMH are susceptible to physiological arousal by lactate infusion similar to patients with panic disorder. To characterize this response further, rats equipped with femoral arterial and venous catheters were chronically infused with L-alalyglycine (L-AG, active isomer whose metabolite blocks glutamic acid decarboxylase, GAD) or D-alalyglycine (D-AG, inactive isomer) via Alzet mini-pumps implanted unilaterally in the DMH. Infusion of 0.5N sodium lactate (10 μl/Kg, i.v.) elicited significant increases in heart rate and blood pressure after 4, 7 or 14 days only in rats with L-AG pumps and not D-AG pumps. Rats with L-AG pumps also showed increased"anxiety" in the plus-maze and social interaction tests as well as decreased GABA content in the DMH when compared to rats with D-AG pumps. These results further strengthen this animal model of panic disorder. (Supported by MH 45362)

PLASMA PROTEIN PATTERN VARIATIONS IN SCHIZOPHRENIA. A. Brett Larive, Dale M. VanderPutter, Carl B. Merri*, Laboratory of Biochemical Genetics, NIMH, Washington, DC 20032 and Monoclonetics Inc., Houston, TX 77027. Plasma protein patterns in 2-D gels from monozygotic twins discordant for schizophrenia were found to be significantly less alike than the protein patterns of normal monozygotic twins and monzygotic twins concordant for schizophrenia. The ELSIE 5-2 D gel analysis program found significantly (p<0.01) fewer protein spot matches between discordant monzygotic schizophrenia twins than either normal monozygotic twins or concordant monzygotic schizophrenia twins. The relative silver stained intensity of matched plasma proteins was also found to vary to a greater extent between monzygotic twins discordant for schizophrenia. Several polypeptide spots were elevated only in the schizophrenic twin. One of these proteins was also found to be significantly (p<0.0001) elevated in the plasma of unrelated schizophrenic individuals (n=75). Additionally, we found differences in plasma haptoglobin levels between monzygotic twins discordant for schizophrenia suggesting variations in liver metabolism.

There is increasing evidence that abnormal brain development is involved in schizophrenia. Among several factors, growth factor responses may play critical roles in brain developmental processes: neuronal proliferation, maintenance, survival and maturation. Since molecular mechanisms of their mitogenic actions are common across cell types, cultured skin fibroblasts were used to conduct these studies. The mitogenic responses of BFGF and EGF were examined in fibroblasts from first-episode, drug-naive psychotic patients (N=10) and normal controls (N=10). Known number of fibroblasts were first synchronized by serum starvation for 24 hr. and then growth factors were added in serum containing medium, and growth was continued. The final number of cells was counted on 7th day. Growth response was determined as % change in cell number. As expected, fibroblasts from normals showed increased mitogenic response (mean±SEM; bFGF=151±5.97, EGF=192±11.99). However, the mitogenic response was reduced in fibroblasts from patients (bFGF=85±6.44, P<0.001; EGF=144±4.74, P<0.04). Further studies on underlying receptor-mediated processes will be presented.

370.12 UPTAKE OF ESSENTIAL FATTY ACIDS IN SKIN FIBROBLASTS FROM SCHIZOPHRENIC PATIENTS. N. S. Shendarkar, S. Mukherjee, J. Mahalik and S. P. Mahalik,* Dept. of Psychiatry and Health Behavior, MCG & VAMC, Augusta, GA 30912.

A generalized abnormal plasma membrane phospholipid (PL) metabolism exists in schizophrenia. Abnormality is predominantly in the distribution of esterified polyunsaturated fatty acids (EPFUs) of essential fatty acids (EFAs: linoleic, LA, and linolenic, ALA). The underlying mechanisms are unclear. The cellular uptake of EFAs and conversion to PUFA(s) are critical for the quantity and quality of PLs. It is difficult to investigate these processes in vivo or using easily available tissues or cells in vitro. Cultured skin fibroblasts are well suited for this. The uptake of EFAs in fibroblasts was compared in three schizophrenic patients with normal controls matched for age, sex and race. Cultures were synchronized by serum starvation for 24 hr. and then grown in serum containing medium for next 24 hr., and fatty acid uptake was determined as moles/mg protein for 30 min. The uptake of both the fatty acids was normal in fibroblasts from patients with LA:ALA ratio of 1:1 (means ± SEM for LA, P=139±526.54, N=108.3±22.77, for ALA, P=53±480.7, N=54.5±78.3) and 20:1 (LA, P=504.3±192.33, P=317.4±19.49 and for ALA, P<31.32, 11.1, N=15.83±5.81). Data indicate that reported lower levels of membrane fatty acids may be a result of defective conversion into EPFAs and incorporation into functional lipids.


There is evidence that infectious and/or abnormal neural-immune mechanisms may be involved in schizophrenia. To evaluate this possibility, 6 measures sensitive to neural-immune activities were assessed in the CSF of 21 male patients (mean age: 41) with schizophrenia (SCCH) in comparison to 16 healthy controls (HC) (mean age: 35). Quinolinic acid (QUIN), a neurotransmitter, was measured by GCMS, and interleukins (IL1B, IL2, and IL6), tumor necrosis factor alpha (TNF alpha) and beta 2 microglobulin (B2MG) by double antibody ELISA.

Mean QUIN levels in SCH were increased by 24% over those in HC (18.17 vs 15.06) respectively, P<0.04 (Chi square). Mean IL6 and B2MG levels did not differ between SCH and HC (2.4 vs. 2.234/ml and 1.3 vs. 1.4/ml respectively). Levels of IL1B, IL2, and TNF alpha did not differ between SCH and HC since 82 vs88; 1009 vs100, and 719 vs100, respectively, had levels below the lower detection limits of the assay.

The relatively small 25% increase in QUIN levels in SCH is less than the 200-500% increase seen in early stage HIV infected patients. The data suggest that a strongly active and abnormal neural-immune process is not occurring in the large majority of these chronic SCH patients. Supported by MH65463, the Stanley Foundation and WHVAMC Schizophrenia Center.


Cyclic AMP (cAMP) accumulation was evaluated in EBV-transformed human B-lymphocyte cell lines from normal and schizophrenic individuals. Each cell line showed characteristic individual responses to PGE1, a prostaglandin agonist, isoproterenol (iso), a β-adrenergic agonist, phorbol 12-myristate-13 acetate (PMA), an activator of protein kinase C (PKC) and staurosporin (STP), an inhibitor of PKC. PGE1, significantly elevated cAMP accumulation. Iso produced a slight but non-significant increase in cAMP accumulation. Pretreatment of the cells with PMA (10-7 M) significantly enhanced cAMP accumulation following treatment with Iso. STP reduced the potentiation of cAMP accumulation produced by PMA plus Iso (1 μM). In contrast, STP enhanced cAMP accumulation following treatment with PGE1 (10-7 M) plus PMA. Cell lines from controls showed a significantly greater cAMP response following treatment with PGE1, plus PMA compared with the cell lines from schizophrenic patients. These data suggest that a deficit may exist in the PKC and/or PGE1 response systems in schizophrenic patients.


Plasma homovanilliac acid (pHVA) levels were examined in 17 patients during their first episode of psychosis before initiating neuroleptic treatment and in 8 normal controls. The effect of neuroleptic treatment was assessed after 3 weeks "doctor's choice" treatment. The mean age of patients was 22.88 yrs and the mean duration of psychosis at entry to protocol was 4.44 days (range 2-10). In all, 48 patients met DSM-IIIIR criteria for schizophrenia. Baseline pHVA (8.65±2.4 mg/ml) and post-treatment pHVA (8.42±2.7 mg/ml) both were significantly lower in patients than in normal controls (16.53±6.6 mg/ml) (P<0.001 for both comparisons). Change in pHVA levels was not correlated with change in clinical state of patients. Patients with negative symptoms were unchanged, but pHVA in early age of onset postmortem studies showing chronic presynaptic dopaminergic underactivity in early onset patients. In summary, our findings indicate that, in some patients, pHVA is low at the very onset of psychosis and this is associated with a suboptimal early response to neuroleptic treatment.


Neurotensin (NT), an endogenous neuroleptic tridecapeptide, and its metabolites were quantitated in CSF collected at 8 NYC patients presenting with schizophrenia. CSF was placed into enzyme inhibitors on ice and 8-12 ML was subjected to HPLC and RIA using H- and C-terminal antibodies. NT added to CSF was recovered in good yields. Direct injection of patients met below 111K criteria for schizophrenia. Baseline pNT (8.65±2.4 mg/ml) and post-treatment pHVA (8.42±2.7 mg/ml) both were significantly lower in patients than in normal controls (16.53±6.6 mg/ml) (P<0.001 for both comparisons). Change in pHVA levels was not correlated with change in clinical state of patients. Patients with negative symptoms were unchanged, but NT in early age of onset postmortem studies showing chronic presynaptic dopaminergic underactivity in early onset patients. In summary, our findings indicate that, in some patients, pHVA is low at the very onset of psychosis and this is associated with a suboptimal early response to neuroleptic treatment.
730.17

Chronic use of antipsychotic drugs produces therapeutic effects on psychosis, concurrent side effects, and neurochemical changes in selected transgenic systems. Much is known about the chronic effect of these drugs on D1 and GABA receptors, dopamine (DA) turnover, and DA neuronal activity. However, the integrated functional effect of chronic antipsychotic drug treatment on the human brain is less well studied, as are the consequences of its withdrawal. We report here results of a within-subject study of regional cerebral glucose metabolism (rCMRGlu) with chronic haloperidol (HAL) in three different states: on HAL treatment, after a 5-day withdrawal, and after 30 days in a drug-free state. Differences between the on-HAL state and the drug-free (30 day) state were found to be the elucidate and putamen (8% and 7% increase, respectively), the thalamus (9% increase), frontal cortex (4% decrease) and cingulate (6% decrease). At the five day withdrawal time, based on our previous study of supersensitivity phenomena in patients, we had predicted rCMRGlu findings consistent with supersensitivity. Examples from representative structures are presented in the Table. After five days of withdrawal, no significant changes from the on-HAL scan were apparent in rCMRGlu in the brain areas analyzed. These data failed to functional metabolic evidence of withdrawal supersensitivity.

730.18
EFFECTS OF THE NMDA ANTAGONIST KETAMINE ON CEREBRAL BLOOD FLOW PATTERNS IN SCHIZOPHRENIC PATIENTS. A.C. Lesh*†, H.H. Hoehn, M. Zhao, D. Medoff*, R.P. Dansky, G.A. Tamminga, M.P.R.C. University of Maryland, Baltimore, MD 21228 and The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

To evaluate glutamatergic transmission in the pathophysiology of schizophrenia, we have studied the action of the NMDA antagonist ketamine in symptomatic individuals. Research protocols were approved by the University of Maryland IRB. Three subanesthetic doses of ketamine (0.1, 0.3 and 0.5 mg/kg) and placebo were given to 12 stable blind injection study. Ketamine worsened mental status 20 minutes after injection in a dose sensitive fashion, selectively on positive psychotic symptoms. No significant between regional cerebral blood flow changes in medicated schizophrenic patients given acute ketamine (0.3 mg/kg). Three 15O water cerebral blood flow PET scans were collected for drug administration and seven scans, at timed intervals, after ketamine. Data were analyzed using Statistical Parametric Mapping (Friston, 1991). The three baseline scans were combined and compared with the two most proximal post infusion scans from three subjects. The brain regions significantly affected by ketamine were the cingulate cortex and dorsomedial thalamus. Hippocampal activity elevations were also evident in individuals. Ketamine-induced activity changes in the cingulate and thalamus may partially account for its psychotomimetic effects.

731.1
INTRACEREBRAL TRANSECTION USING CATIONIC SYNTHETIC LIPID: AN ALTERNATIVE TO VIRAL VECTOR IN C5 gluMOA "SUICIDE GENE": THERAPY. P. Fujita*, IM. Veinor*, D. Cappari*, D. Chen, G. Amalfitano, IF Brunet, I. Motin, MF Nisou*, IM. Venet, AL. Berhad*, INSERM U 318, BP 217X GRENOBLE 38043 Cedex 9, FRANCE.

Therapeutical trials have been begun in human gliomas using retroviral transfection. It confronts us with the ethical problem of the use of vector obtained from infectious agents. For these reasons we investigate in vivo transfection using the synthetic vector DOTAP. We used the "suicide gene" Herpes thymidine kinase (TK). It transforms the antiviral ganciclovir in toxic phosphorylated components and induces a bystander-effect which explains that no more than 10 % of the cells need to be transfected.

DOTAP injected in normal rat brain did not induced any histological damages. In a model of C5 glioma cells transfected with TK and treated with GC was observed. A single stereotaxis TK transfection induced focal necrosis and no more than 50% volume reduction. Then, we used intratumoral cannulae providing intratumoral targeting and repeated transfections. In these conditions we observed dramatic tumor regression. When TK was injected outside the tumor we did not observed histological damages in the brain itself contrasting the absence of toxic effect of TK transfection in non dividing cells.

These results suggests that the DOTAP vector is as efficient as retroviral vector in a "suicide gene therapy" for experimental gliomas. It confort a phase I study in humans using repeated transfections through a nickham intra-tumor delivery.

731.3
A NOVEL HERPES SIMPLEX VIRUS "PIGGY BACK" PACKAGING SYSTEM FOR AMPLICON VECTORS. Peter Peñas*, I. Antonio Chico*, N. Antonio Chico*, Richard Thompson*, David P. Cooke*, and Xavier O. Breakefield. Dept. Neurology and Neurosurgery, Massachusetts General Hospital, Harvard Medical School, Boston MA 02114; Dept. Mol. Genetics, University of Cincinnati Medical Center, Cincinnati, OH 45267.

Herpes virus type I (HSV-1) vectors have proven to be efficient at transgene delivery to neurons and other cell types in culture and in vivo. In the recombinant virus system transgenes are incorporated directly into the HSV-1 genome. In the ampiclon system bearing the HSV-1 genome in one packaging vector, and a packaging signal, as well as a transgene, is packaged into virions through the aid of a helper virus. These two systems can be combined by making propagation of the vector and helper viruses mutually dependent on each other. In this study we are characterizing an ampiclon "piggy-back" vector system that uses a viral promoter to express a gene essential for HSV-1 replication, propagated with a mutant virus deleted in the same replication-essential gene. We have constructed ampiclon vectors bearing the immediate early IC44 gene under the control of the SV40 and the gial specific JC virus promoter elements. These promoters can be used to regulate expression of both IC44 and another gene in one ampiclon using an 15RS translational read-through element. As a helper virus we have used the IC44 deletion mutant d120 from Dr. Neil DeLuca, Univ. Pittsburgh, propagated on Vero cells or 207 glioma recombinants was selected using by PCR and Southern blots of Vero plaque lysates. Current data indicate that this novel packaging system has markedly delayed cytotoxic effects as compared to wild type virus, and is a more efficient delivery to neurons. This system provides a basic model which can be modified to include cell-specificity of vector propagation and inclusion of multiple transgene elements.

731.4

Herpes virus mutants offer a potential therapeutic alternative to conventional treatment modalities for malignant brain tumors. The rationale for this approach is that these genetically mutant viruses replicate preferentially in tumor cells and not within cells of the nervous system, which are generally post-mitotic. Thus, they selectively lyse tumor cells within the CNS. We have used a neuroattenuated Herpes Simplex Virus type 1 (strain 1716F) for in vitro and in vivo studies. In vitro, HSV-1716F is efficient at lysing human NTera-2 (a teratocarcinoma cell line) cells and two medulloblastoma lines DAOY and D283. In vivo, HSV-1716F regresses human NTera-2 brain tumors in nude mice and prolongs their survival relative to mock treated, tumor bearing mice. We have studied the pathology over time associated with tumor regression using Magnetic Resonance Imaging and immunohistochemical localization of HSV and tumor cells. We have also observed that viral replication is limited to the tumor as predicted. Thus, our results suggest that this is a feasible approach for the treatment of malignant brain tumors.
Long-term Observations of Thymidine Kinase Deficient HSV-1 Infection in the Rat Brain

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We have been using Herpes simplex virus to deliver foreign genes into the nervous system and to treat brain tumors. As a human pathogenic virus, the HSV-1 in its wild type form has been well known to cause clinical symptoms including severe encephalitis. We have recently demonstrated the effectiveness of the HSV-1 viral vectors in both gene expression and tumor-cell killing. In the present study, we have investigated pathogenic effects of the viral vectors in animals that survived at least 18 months after treatment. Rats were intracerebrally injected with 1 µl (10^8 pfu) of KOS-SB, a thymidine kinase-defective HSV-1 mutant (tk(-) HSV-1). Animals that survived at least 18 months without showing any sign of illness before sacrificed, and brain tissue was sectioned for histological and immunocytochemical observations. Neuronal stains showed a significant enlargement of the lateral ventricles in both hemispheres. Otherwise, the histology of other brain regions appeared normal. Immunocytochemistry with a polyclonal antibody for the HSV-1 virus failed to detect any viral antigen in these long-term surviving animals while positive immunostaining could be seen in brains of animals 7 days after viral injection. In these "short-term" animals, cells in the paraventricular regions showed strong reaction to the antibody staining. Monoclonal antibodies against major histocompatibility complex (MHC) antigens were also used to detect possible immune and inflammatory responses to the viral infection. No immunopositive cells were found in brains of the long term rats. Our results suggest that, although intracerebrally injected tk(-) HSV-1 virus caused mild encephalitis, the damage was limited and long term effects were minor. The failure to detect antigens of both the virus and MHC in long term animals further suggests that any damage observed resulted from an acute response rather than a chronic effect.

ADENOVIRUS-MEDIATED GENE THERAPY FOR EXPERIMENTAL PRIMARY AND METASTATIC BRAIN TUMORS

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The therapeutic efficacy of adenovirus-mediated transduction and ganciclovir (GCV) administration was tested in models of primary and metastatic brain tumors. A replicative-defective recombinant adenovirus vector (ADV-tk) was created that contained the herpes simplex virus thymidine kinase (HSV-tk) gene controlled by the Rous sarcoma virus LTR. HSV-tk phosphorylates GCV which acts as a chain terminator of DNA synthesis and selectively kills dividing cells. Syngeneic tumors were generated by injection of cultured glioma (9L) or breast tumor cells (MAT-B) cells into the rat caudate. Eight days after tumor cell injection, ADV-tk or a control adenovirus carrying the β-gal gene was injected into the tumors. The animals then were treated twice daily for 6 days with GCV or saline. Twenty days after tumor cell injection, the brains were examined microscopically and tumors were measured. Control rats in both models had large tumors. Rats treated with ADV-tk and GCV had no tumors at the primary site. Necrosis with macrophage and lymphocyte infiltration was present. In survival tests, control rats lived only 22 days after 9L cell injection. Treated rats lived 126 days when they were examined and found to have no residual tumors. These results demonstrate that adenovirus-mediated transfer of the HSV-tk gene confers cytotoxic sensitivity to GCV to tumors in vivo.